201-14221

To: Oppt.ncic@epamail.epa.gov
cc: Jane Vergnes <JVergnes@ispcorp.com>, Christopher Bradlee <bradlec@basf-corp.com>

Subject: HPV Submission CASNO 96-48-0

Attached is the HPV submission for gamma-Butyrolactone, CASNO 96-48-0. There are three attachments in pdf format:

1. Cover letter
2. Test plan
3. Robust summaries

This submission is made on behalf of the BPPD Consortium (reg no

Please call or email me if you have any difficulty receiving or opening the submission.

Elmer Rauckman PhD DABT

rauckman@toxicsolutions.com

618-539-5280

96-48-0-CL.pdf 96-48-0-TP.pdf 96-48-0-RS.pdf

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Elmer Rauckman, Ph.D. DABT
Toxicology and Regulatory Affairs

1201Anise Court
Freeburg, IL 62243

Phone: (618) 539-5280 rauckman@toxicsolutions.com Fax: (618) 539-5394

December 30, 2002

Christine Todd Whitman
US Environmental Protection Agency
PO Box 1473
Merrifield VA 22116

Re: Submission of γ-Butyrolactone (CASNO 96-48-0) Documents

Via Electronic Submission to Oppt.ncic@epa.gov

Registered with EPA as:
BPPB Consortium, **Registration Number**

Dear Administrator Whitman;

On behalf of the γ-Butyrolactone Consortium, I am submitting the attached test plan and robust summaries for γ-Butyrolactone (CASNO 96-48-0), submitted under the United States Environmental Protection Agency's High Production Volume Chemical Challenge Program. This submission consists of a test plan and a set of robust summaries for this material.

The Consortium members sponsoring this submission are

- BASF Corporation
- International Specialty Products

This document is being submitted in electronic format (Adobe Acrobat pdf files). If you require additional information or have problems with the electronic document please contact me as a representative of the Consortium by phone (618-539-5280) or email (erauckman@charter.net).

Sincerely,

Elmer Rauckman, PhD, DABT
Consulting Toxicologist

Attachments:

Testing Plan	96-48-0-TP.pdf
Robust Summaries	96-48-0-RS.pdf

CC: BASF
ISP

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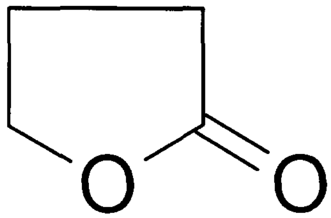
γ-Butyrolactone



CAS Number 96-48-0

U.S. EPA HPV Challenge Program Submission

December 30, 2002

Submitted by:

γ-Butyrolactone Consortium

Prepared by:
Toxicology and Regulatory Affairs
1201 Anise Court
Freeburg IL 62243
618-539-5280

Table of Contents

Executive Overview

γ-Butyrolactone is a water-soluble oily liquid, with a boiling point of 206° C. It has a vapor pressure of 0.344 hPa at 20° C and a log Ko/w of -0.64. It is used as a chemical intermediate, as a solvent for polymers, in some paint removers, and in some printing inks.

γ-Butyrolactone was found to be readily biodegradable and has an estimated indirect photolysis half-life in the atmosphere of 5.5 days. It is labile to hydrolysis by water, especially at high pH levels, giving γ-Hydroxybutyrate as a product. In water at low pH, it can exist as an equilibrium mixture of γ-Butyrolactone and γ-Hydroxybutyrate. γ-Butyrolactone has a low order of toxicity for fish, daphnids and green algae, and its hydrolysis product is predicted to have even lower toxicity.

γ-Butyrolactone is readily absorbed by the oral or inhalation routes and can penetrate the skin. It is rapidly converted to γ-Hydroxybutyrate in the body and excreted quickly, primarily as carbon dioxide, which shows that it undergoes extensive metabolism. Due to its rapid metabolic conversion to γ-Hydroxybutyrate, which has an effect on the central nervous system, γ-Butyrolactone has a weak narcotic effect. It displays low acute-oral toxicity with an oral LD_{50} for rats in the range of 1500 to 2000 mg/kg-bw. Adverse effects are limited to clinical signs of weakness, unconsciousness, and increased depth of respiration. Other than narcotic action on the CNS, no target organs have been identified. In rats, inhalation of a saturated atmosphere (8 hours at 20°C) did not cause any adverse effects and indicates a low acute-toxic effect of γ-Butyrolactone by inhalation. This observation has been confirmed and extended by mixed vapor/aerosol studies.

Repeated exposure causes no specific effects other than sedation, which animals develop a tolerance to within a few weeks. In a 13-week oral gavage study in rats, the NOAEL was 225 mg/kg for males (based on body weight gain) and 450 mg/kg for females (based on one death in the 900-mg/kg group). No specific target organs were identified. In the companion study in mice, except for minor sedation during the first few weeks of study, the NOAEL was 525 mg/kg for males (based on body weights and mortality), and 525 mg/kg for females (based on one death in the 1050-mg/kg group). No specific target organs were identified.

Two-year carcinogenicity studies were essentially negative and the NAOEL for male or female rats was 225 mg/kg-day. In mice, the NOAEL for males was 262 mg/kg (survival) and the NOAEL for females was < 262 mg/kg (body weight gain). No specific target organs were identified.

Extensive genotoxicity testing has yielded primarily negative results but high concentrations, in the presence of an exogenous metabolizing system caused a positive response in the *in vitro* sister chromatid exchange and chromosome aberration assays. *In vivo* studies were negative.

Fetal weight was significant increased in pregnant female rats treated by gavage on days 6 to 15 of pregnancy. No differences from unexposed animals in the corpora lutea, total implantations, ratio of dead to live fetuses, resorptions, and pre- and post-implantation losses were noted at doses up to 1000 mg/kg. In addition, there were no visceral or skeletal malformations due to γ-Butyrolactone exposure. One-time intraperitoneal dosing during proestrus interfered with FSH and LH production and inhibited ovulation in Sprague-Dawley rats. The relevance of this finding to fertility is unknown.

The critical effect for γ-Butyrolactone appears to be a weak narcotic effect on the CNS, which is an effect also know to occur in humans due to the rapid metabolism of γ-Butyrolactone to the neurologically active γ-Hydroxybutyrate.

It is concluded that the available information adequately fills all the data elements of the HPV. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, conduct of additional similar studies would not add significantly to our understanding of this material's hazard.

Testing Plan and Rationale

Testing Plan in Tabular Format

CAS Number 96-48-0 γ-Butyrolactone	Information Available?	OECD Study ?	GLP Study?	Supporting Information?	Estimation Method?	Acceptable?	Testing Recommended?
HPV Endpoint							
Physical Chemical							
Melting Point	Y	N	N	N	N	Y	N
Boiling Point	Y	N	N	Y	N	Y	N
Vapor Pressure	Y	N	N	Y	N	Y	N
Partition Coefficient	Y	N	N	Y	N	Y	N
Water Solubility	Y	N	N	Y	N	Y	N
Environmental & Fate							
Photo-Degradation	Y	N	N	N	Y	Y	N
Water Stability	Y	N	N	Y	N	Y	N
Transport	Y	N	N	N	Y	Y	N
Biodegradation	Y	N	?	Y	N	Y	N
Ecotoxicity							
48-Hour Fish	Y	N	N	Y	N	Y	N
48-Hour Invertebrate	Y	Y	N	Y	N	Y	N
96-Hour Algae	Y	Y	N	Y	N	Y	N
Toxicity							
Acute	Y	N	N	Y	N	Y	N
Repeated Dose	Y	N	Y	Y	N	Y	N
Genetic Toxicology *in vitro*	Y	N	Y	Y	N	Y	N
Genetic Toxicology *in vivo*	Y	N	Y	Y	N	Y	N
Reproductive	Y	N	N	Y	N	Y	N
Developmental	Y	N	N	N	N	Y	N

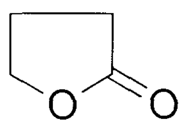
Introduction

γ-Butyrolactone, CAS no. 96-48-0, is a cyclic ester (lactone) most commonly prepared from 1,4-Butanediol by a Reppe process. The reaction is carried out in the gas phase between 180 and 240° C, over a copper catalyst and provides approximately 95% yield. The γ-Butyrolactone is separated from impurities (1,4-Butanediol, Butyric acid and high-boiling oligomers) by distillation giving a commercial product of about 99.7% purity. γ-Butyrolactone is also commercially prepared by the hydrogenation of Maleic anhydride using a nickel catalyst (1). It can also by synthesized from acetylene and formaldehyde under high pressure (2).

γ-Butyrolactone is a clear-oily liquid (3) with a pleasant odor (2). It has low volatility and is miscible with water and most organic solvents (3).

This material has numerous industrial applications due to its chemical structure and solvent properties. The bulk of γ-Butyrolactone production is used as an intermediate in the synthesis of N-Methylpyrrolidone (NMP) and 2-Pyrrolidone. It is also used to manufacture herbicides, growth regulators, vitamin B1, and the rubber additive thiodibutyric acid.

Several diverse solvent applications have been reported including as a solvent for polymers, in hairwave compositions and sun lotions, as a cosolvent for capacitor electrolytes and as a cosolvent for electronic photoresists. It is also used in printing inks, (for example ink-jet printer inks), as an extracting solvent in the petroleum industry. γ-Butyrolactone has also found application as a nematocide (1).



The structure of γ-Butyrolactone is shown above. This material is also known as:

- Dihydro-2(3H)-furanone
- Dihydro-2-furanone
- Butyrolactone,
- 4-Butyrolactone
- 4-Hydroxybutyric acid cyclic ester
- 4-Hydroxybutanoic acid lactone
- Tetrahydro-2-furanone

- 1,2-Butanolide
- 1,4-Butanolide
- 4-Deoxytetronic acid

Exposure in industrial applications is limited by process controls, protective equipment, low vapor pressure and by warning properties due to its odor. No occupational exposure level set by any governmental agency was located. Use as a co-solvent in digital inks may result in a very low-level of inhalation exposure by consumers limited by the very low quantities of ink used by consumer digital printing devices.

Several physicochemical, fate and toxicity studies have been conducted with γ-Butyrolactone. These studies are briefly reviewed in this testing rationale document, which also describes how these studies meet the SIDS (Screening Information Data Set) end-points of the United States Environmental Protection Agency (USEPA) High Production Volume Challenge (HPV) program. Robust summaries have been prepared for key studies; supporting studies are referenced in these summaries or given as shorter summaries using the IUCLID format. The available data set satisfactorily fulfills the data requirements for the EPA HPV Program. The majority of data elements are filled by high-reliability studies on γ-Butyrolactone, where direct data are not available or data are sparse, surrogates and acceptable estimation methods are used to fill or supplement the data element, as encouraged by the U.S. EPA and other regulatory authorities to avoid unnecessary testing cost and animal usage.

Physicochemical Data

Physicochemical data for γ-Butyrolactone are available from the literature and manufacturer's information.

Melting Point	-43.5° C (4)
Boiling Point	204° C @ 1013 hPa (5)
Vapor Pressure	0.344 hPa @ 20° C (6)
Partition Coefficient	Log $K_{o/w}$ = -0.64 (7)
Water Solubility	Soluble in all proportions (5)

Table 1: Physicochemical data for γ-Butyrolactone

These properties indicate that γ-Butyrolactone is a slightly volatile liquid with high water solubility. The value of the partition coefficient suggests that γ-Butyrolactone will partition preferentially into water and, therefore, has little potential for bioaccumulation. The determination of an accurate and representative $K_{o/w}$ of γ-Butyrolactone is complicated by the fact that, in aqueous solution, γ-Butyrolactone can be an equilibrium mixture of γ-Butyrolactone, γ-hydroxybutyric acid, and γ-hydroxybutyrate. The equilibrium is pH and temperature dependent with sub-dependencies of alkalinity and dilution (see water stability discussion). The above value is supported by a value of −0.566 from BASF (8) and is considered representative of the conditions that would be encountered in the environment as it was derived by a "shake-flask" method.

Recommendation: No additional physicochemical studies are recommended. The available data fill the HPV required data elements.

Environmental Fate and Pathways

Biodegradation potential has been determined using the MITI test and a BOD test. In the MITI test, a degradation of 60-92% was reported in 14 days (9), indicating that this material is considered readily biodegradable. In the BOD test with non-acclimated sludge, a removal of >95 % was recorded after 8 days (10). This ready biodegradation is anticipated based on the structure and its hydrolysis product γ-hydroxybutyrate, which is quickly metabolized to carbon dioxide in mammals. The biochemical pathways are well known and the structure is linear.

Photodegradation was estimated using version 1.90 of the Atmospheric Oxidation Program for Microsoft Windows (AOPWIN) that estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. The estimated rate constant is used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radical. The program produced an estimated rate constant of 2.31 E-12 cm^3/molecule-sec. Using the default atmospheric hydroxyl radical concentration in APOWIN and the estimated rate constant for reaction of γ-Butyrolactone with hydroxyl radical, the estimated half-life of γ-Butyrolactone vapor in air is approximately 56 hours (see accompanying robust summary) (11).

Water stability has been thoroughly investigated for this material. In *Ullmann's Encyclopedia of Industrial Chemistry* it is stated that, in water γ-Butyrolactone is an equilibrium mixture of the lactone (closed ring) and hydroxybutyric acid. The equilibrium is pH and temperature dependent. At neutral pH and 0° C, the equilibrium lies 100% on the lactone side; at 100° C, the equilibrium mixture is 80% lactone. In the presence of base, the equilibrium shifts to the acid form, and the equilibrium mixture is 100% acid form in the presence of 1 equivalent of base. These observations have been quantitatively refined in a recent study published it the *Journal of Forensic Science* in which the equilibrium was investigated in pure water and in buffered water at several pH values (12). The results show that γ-Butyrolactone is relatively stable in pure unbuffered water. A 0.5% solution takes about 120 days to reach a stable pH of approximately 3.3 and equilibrium of 67% lactone to 33% acid form. This mixture was stable for at least 100 days after reaching steady state. In neutral buffered solutions, the hydrolysis is more rapid 15-30 day half-life and proceeds to completion (97% measured after 202 days). Additional studies showed that at higher pH values the hydrolysis is faster and at pH 12 , it proceeds to completion in about 10 minutes. At more acidic pH levels, it is slower and a 0.5% solution at pH 2 reaches an equilibrium of about 2:1 lactone to acid form. In addition, if the initial 0.5% solution at pH 2 starts in 100% of the acid form, it still reaches the same equilibrium ratio in about the same time frame. Studies were not conducted to determine the effect of concentration on hydrolysis, but it can be predicted from the hydrolysis equation that dilution will increase the proportion of acid form by dilution of the hydrogen ion concentration. It is concluded that the water stability is well characterized.

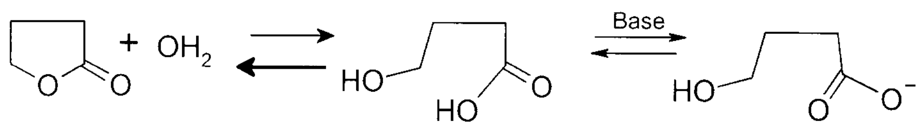


Figure 1: Hydrolysis of γ-Butyrolactone

Theoretical Distribution (Fugacity) of γ-Butyrolactone in the environment was estimated using the MacKay EQC level III model with standard defaults in EPIWIN v 3.05 but using the measured vapor pressure of 0.259 mm Hg, the measured log Ko/w, and data-adjusted estimates for half-life in water, soil and sediment. (13). The results for distribution using a model calculated Ko/c (adsorption coefficient based on organic carbon content) of 0.0939 and equal initial distribution to air, water and soil are:

Air	3.2 %
Water	34.3 %
Soil	62.4 %
Sediment	0.02 %

Table 2: Theoretical Distribution (Fugacity) of γ-Butyrolactone in the environment

Recommendation: No additional fate and pathway studies are recommended. The available data fill the HPV required data elements.

Ecotoxicity

An unpublished study of the acute toxicity of γ-Butyrolactone to the freshwater fish *Leuciscus idus* showing a 48-hour LC_{50} of 275 to 302 mg/L (14) indicates that this material presents little acute hazard to freshwater fish. A daphnia study indicates an EC_{50} greater than 500 mg/L (15). Green algae tests indicate an IC_{50} of 79 mg/L (16). These values, with references, are shown in the table along with results of ECOSAR modeling using the "Esters" model, based on the measured $K_{o/w}$ of –0.64. The measured data appear to fit the ECOSAR "Esters" model well.

Aquatic Toxicity of γ-Butyrolactone		
	Reported Values	ECOSAR Prediction
Fish, 48 hour LC_{50}	275-302 mg/L (14)	334 mg/L*
Daphnia, 48 hour EC_{50}	> 500 mg/L (15)	17300 mg/L*
Algae, 96 hour EC_{50}	79 mg/L (16)	24 mg/L*

* Estimated using ECOSAR with measured $K_{o/w}$ (17)

Table 3: Aquatic Toxicity of γ-Butyrolactone.

Two issues are potential confounders in these aquatic studies. The first is volatility; however, based on the vapor pressure and water solubility (Henry's Law Constant) of the test material, this is not considered to be a concern in these short-term studies.

A more important consideration for γ-Butyrolactone is the water stability of the test material. Data show that this material converts to γ-Hydroxybutyrate in neutral and basic solution. The kinetics of this hydrolysis are well established (12) and at pH 7 it is known there is only about a 15% hydrolysis in 3 days. Based on the known pH dependency, it can be extrapolated that the 48-hour loss in the pH 7-8 range for the fish and daphnid studies was less than about 20%. The impact of this conversion to γ-Hydroxybutyrate can be evaluated by comparing the aquatic toxicity of γ-Butyrolactone with that of γ-Hydroxybutyrate. No measured aquatic toxicity values could be located for γ-Hydroxybutyrate; however, its toxicity was estimated with the ECOSAR modeling program. The estimated fish 96-hour LC_{50} is 13,900 mg/L and the estimated Daphnia EC_{50} is 12,600 mg/L (18). Thus, the impact of the hydrolysis product γ-Hydroxybutyrate on the aquatic toxicity results for fish and daphnids is considered minor.

For the longer-term algae studies, however, where the pH of inoculated flasks containing the lower concentrations of test material exceeded 10.0 at the 96-hour interval, the loss of test material may be in excess of 50%. In this case, the solution by the end of the algae studies could have contained 50% or more of the test material as γ-Hydroxybutyrate. No measured algal inhibition values could be located for γ-Hydroxybutyrate to determine its potential impact on the result; however, its algal toxicity was estimated with the ECOSAR modeling program. The estimated 96-hour IC_{50} for green algae is 68,800 mg/L. The impact, therefore, would be to reduce the apparent inhibition of the test material. This happens to be in agreement with the observed IC_{50} of 79 mg/L as compared to the model-calculated, or "expected", value of 24 mg/L. The value of 24 mg/L is considered more representative of the 96-hour green algae IC_{50} for γ-Butyrolactone. On the other hand, since the realistic situation is that hydrolysis will be occurring and as no "flow through" system is available for algae studies and as the hydrolysis kinetics of γ-Butyrolactone are well understood; the value is know with sufficient accuracy for the purposes of the HPV assessment.

Recommendation: No additional ecotoxicity studies are recommended. The available data fill the HPV required data elements. The data are also consistent with the ECOSAR model and with available hydrolysis data.

Health Effects

Several studies have been conducted to estimate the potential health effects of γ-Butyrolactone to man. These span from acute studies (by all potential routes of absorption) to lifetime studies in rats and mice. In addition several studies have carefully examined the reproductive organs after various exposure durations and found no effects. Developmental toxicity using the recommended maximum dosage for experimental animals has been conducted. Since γ-Butyrolactone was part of an inter-laboratory collaborative program to improve genotoxicity testing, it has a wealth of high-quality genotoxicity studies available. ADME studies are also available defining its toxicokinetic characteristics. Few systemic adverse effects have been associated with the administration of γ-Butyrolactone to experimental animals. The most important effect involves its rapid metabolism to γ-Hydroxybutyrate, which is active in the CNS producing sedation and other higher-level effects on dopaminergic and GABAnergic neural pathways. This section summarizes the available data and assesses potential health effects from exposure to γ-Butyrolactone.

Metabolism

Adsorption, distribution, metabolism and excretion are important components in developing an understanding of the potential health effects of a material and of extrapolating data between studies, between routes of administration and between compounds. In the case of γ-Butyrolactone a considerable amount of information has been generated.

Absorption by the oral route has been determined to be both rapid and complete with a peak plasma concentration after dosing proportional to the dose. The completeness of absorption by the oral route is supported by the observation that after oral administration, if total plasma concentration of the compound and its principal metabolite, γ-Hydroxybutyrate, is plotted against time, the area under the curve is nearly identical to that following intravenous administration of γ-Butyrolactone (19, 20). Dermal dosing has been estimated to result in about ten percent absorption of γ-Butyrolactone (21).

Butyrolactone is metabolized rapidly with elimination primarily via respiratory CO_2 and urinary metabolites. Roth and Giarman reported that after a single intravenous dose of ^{14}C labeled γ-Butyrolactone to rats, traces of $^{14}CO_2$ were detectable in respiratory air after only 4 minutes, and reached a maximum in 15 minutes. Sixty percent of the total radioactivity was eliminated as carbon dioxide in less than 2.5 hours (22, 23). The plasma half-life of intravenously administered γ-Butyrolactone is less than one minute in rats. It has been reported that γ-Butyrolactone is converted to γ-Hydroxybutyrate by a "lactonase" enzyme present mainly in the plasma and liver; enzymatic activity was not detected in other tissues including brain, kidney, heart, skeletal muscle, and intestine. A γ-lactonase catalyzing the formation and hydrolysis of four- to eight-carbon lactones has been purified from human blood and has similar kinetic properties to that isolated from rat liver microsomes (24).

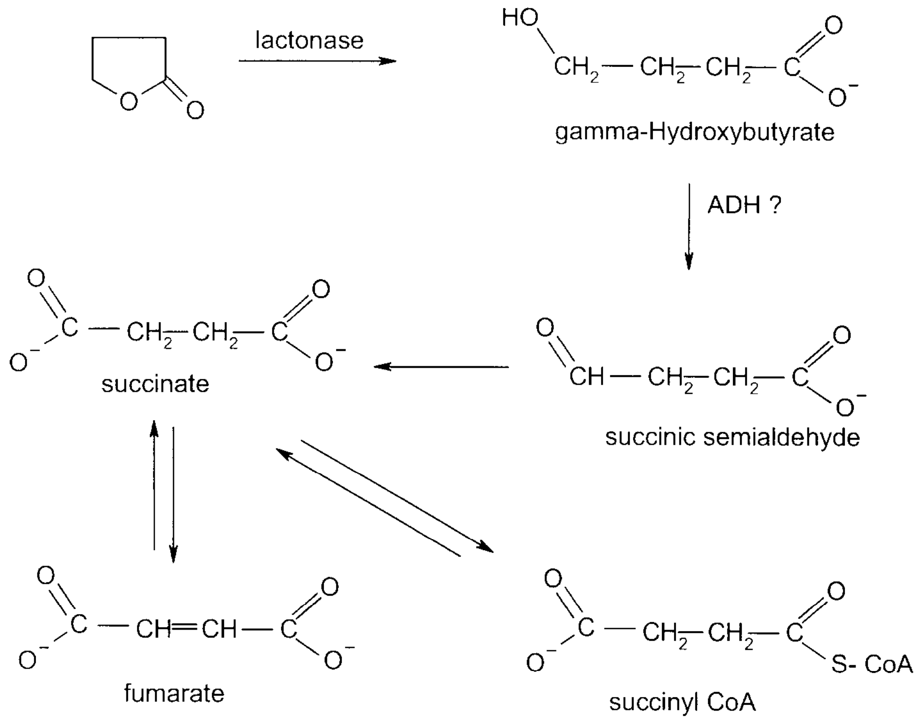


Figure 2: Proposed Metabolic Pathway of γ-Butyrolactone

The pathway from γ-hydroxybutyrate to carbon dioxide is controversial (25) with early work suggesting that the tricarboxylic-acid cycle was primarily involved via succinate (26). Later investigators obtained substantial labeling of succinate and its amino acid metabolites in the brain of rats after intraventricular administration of [1-^{14}C]-labeled γ-Hydroxybutyrate (27). In addition, demonstration that the labeling pattern in the mouse brain after an intravenous injection of [1-^{14}C]-labeled γ-Hydroxybutyrate can be explained by oxidation via succinate, but not by ß-oxidation, eliminates beta-oxidation as a probable pathway (28). Data showing that γ-Hydroxybutyric acid is metabolized to γ-aminobutyric acid in incubated brain slices and that specific inhibitors of γ-aminobutyrate-2-oxoglutarate transaminase blocked the production of labeled γ-aminobutyric acid from labeled γ-Hydroxybutyric acid and of labeled 2-oxoglutarate from labeled glutamate, suggested that the catabolism of γ-Hydroxybutyric acid to γ-aminobutyric acid occurs via a transamination mechanism and not through the Krebs cycle (29). In spite of the transaminase pathway possibly having importance in the neurologic effects of γ-Butyrolactone, the rapidity of excretion and low degree of toxicity argue that a more generalized mechanism (such as intermediary metabolism and/or Krebs cycle) is prevalent. More recent work by Gibson and Nyhan (30) has shown that homogenates of liver and kidney mitochondria, but not heart, readily converted [U-^{14}C]-γ-Butyrolactone to ^{14}C organic acids via a pathway of conversion to ^{14}C-succinic acid, followed by further metabolism through the tricarboxylic acid cycle. Furthermore, this conversion was facilitated by exogenous NAD^+ and $NADP^+$. No evidence for the beta-oxidation of γ-Butyrolactone was obtained in any of the mitochondrial sonicates. Studies with exogenous non-labeled succinic semialdehyde indicated that this compound is an intermediate in the conversion of γ-Butyrolactone to succinic acid. Further evidence that succinate is involved in the metabolism of γ-Butyrolactone comes from the finding that patients with the rare genetic defect leading to succinic semialdehyde dehydrogenase deficiency (SSADH), accumulate 4-hydroxybutyric acid in physiologic fluids (31). Based on these findings and considerations, the figure above presents the proposed primary initial metabolic pathways for γ-Butyrolactone. After conversion to succinate and fumarate, it enters intermediary metabolism where it is driven to carbon dioxide by dose-dependent mass balance. This rapid conversion into labile components of intermediary metabolism and the Krebs cycle is considered to account for its low systemic toxicity.

This proposed pathway is in accord with the known low systemic toxicity of succinic acid and offers a logical explanation for the low degree of γ-Butyrolactone toxicity to mammals. This metabolic understanding adds to our confidence in hazard and risk assessment for γ-Butyrolactone.

Acute Toxicity

Oral Exposure

Multiple determinations of the oral LD_{50} of γ-Butyrolactone have been reported in the rat, mouse, rabbit, cat and guinea pig (32); the studies universally indicate a low order of acute oral toxicity for this material. Two robust summaries have been prepared from representative studies in rats. One that used seven dose groups, provides an oral LD_{50} in the rat of 1920 mg/kg (33). A supporting study has also been summarized that found an oral rat LD_{50} of 1580 mg/kg (34). An overview of the studies indicates that a consistent narcotic or sedative effect at dose levels of approximately 500 mg/kg and above has been reported. Depending on the dose and the species, narcosis lasts from a few minutes to several hours but surviving animals show no other adverse effects or specific target organ effects.

Inhalation Exposure

It has been reported that there were no deaths when rats were exposed to saturated vapor of γ-Butyrolactone for 8 hours (34). The actual concentration was not measured but based on the vapor pressure at the vapor concentration is calculated to be in the range of 300 ppm. Other investigations have reported LC_{50} values of > 2680 mg/m^3 (35) and > 5100 mg/m^3 (36); clinical signs were exophthalmus, difficulty breathing, hypoactivity and temporary reduction in food intake but no adverse effects were found at necropsy.

Dermal Exposure

One study in guinea pigs was found in the literature that indicated the dermal LD_{50} of γ-Butyrolactone is 5640 mg/kg (37).

Recommendation: No additional acute toxicity studies are recommended. The available data fill the HPV required endpoints for acute toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, the weight of evidence shows that the oral and inhalation toxicity is very low. Likewise, the limited study of dermal toxicity provides support for low hazard by this route. Conduct of additional studies would not add significantly to our understanding of this material's toxicity and it is recommended that no additional acute toxicity studies be conducted.

Repeat Dose Toxicity

Oral Exposure

Definitive oral subchronic gavage studies have been conducted in both rats and mice by the U.S. National Toxicology Program (25). In the rat studies, groups of 10 rats of each sex received test material by gavage at doses of 0, 56, 112, 225, 450, or 900 mg/kg of body weight, 5 days a week for 13 weeks. All high-dose males and one high-dose female died. Males receiving 450 mg/kg gained less body weight. There was no body-weight effect in females at any dose level. Other than inflammation of the nasal mucosa in all groups of dosed rats, there were no specific organ effects. The nasal mucosa irritation was considered to be a non-specific effect of gavage with a volatile agent. Rats at the higher dose levels (225 mg/kg and above) showed signs of sedation after dosing during the first 2-3 weeks of study that diminished in intensity with continued dosing, and dosed rats showed no visible signs of sedation after three weeks of dosing. The NOAEL was 225 mg/kg for males (based on body weights), and 450 mg/kg for females (based on one death in the 900-mg/kg group). No specific target organs were identified.

In the mouse studies, groups of 10 mice of each sex received test material by corn-oil gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg five days a week for 13 weeks. Groups of 10 mice received test material by corn-oil gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg five days a week for 13 weeks.

In addition to these subchronic exposures, 2-year studies have also been conducted by oral gavage. In the rat studies, groups of 50 rats of each sex were administered γ-Butyrolactone in corn oil by gavage five days a week for up to 103 weeks. Male rats received 0, 112, or 225 mg/kg, female received 0, 225, or 450 mg/kg of body weight. In male rats there was no body weight change associated with administration of 112 or 225 mg/kg-day test material. Likewise, there was no apparent adverse effect of the test substance on survival as there was a marginal increase in survival of high-dose males. This was attributed to a marginal decrease in mononuclear cell leukemia in the high-dose males. There were no nonneoplastic toxic lesions or increased incidences in neoplasms in dosed male rats that were attributed to the administration of γ-Butyrolactone.

In the 2-year mouse studies, groups of 50 mice of each sex were administered γ-Butyrolactone in corn oil by gavage 5 days a week for up to 103 weeks. Both male and female mice received 0, 262, or 525 mg/kg of body weight. Mean body weight and survival of high-dose male mice were significantly lower than in controls. High-dose mice were partially sedated or lethargic and inactive shortly after dosing; this seemed to contribute to an increase in fighting related trauma in dosed males, resulting in lower body weights and excess mortality. After the male mice were individually housed (week 67), the difference between mean body weights of dosed and control groups decreased. Body weights of low- and high-dose female mice were lower than that of the controls throughout much of the study, but there was no improvement following the change to individual housing. Survival of dosed female mice was similar to controls.

Administration of test substance to mice for 2 years was associated with a statistically significant increased incidence of focal hyperplasia of the adrenal medulla in low-dose males but not high-dose males. There were no nonneoplastic degenerative lesions associated with the administration of γ-Butyrolactone to male or female mice.

Recommendation: No additional repeated-dose studies are recommended. The available studies conducted by the NTP Statement if Work Guideline under GLP adequately fills the HPV required data element for repeated-dose toxicity.

Genetic Toxicity

The SIDS/HPV requirement for genetic toxicity screening is for two end-points, one sensitive to point mutation and one sensitive to chromosomal aberrations. In the case of this material, adequate tests have been conducted that cover both of these endpoints.

Genetic Toxicology in vitro

Several adequate *in vitro* tests of genetic toxicity for γ-Butyrolactone are available. A Salmonella typhimurium reverse mutation assay conducted by the NTP is representative and has been prepared as a robust summary (see attached robust summaries)(38). There are several supporting studies that have been published in the literature (39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55). Similarly, there are supporting reverse mutation tests in *E. coli* that show either clear negative results or results that cannot be interpreted (56, 57, 58, 47). Other various DNA damage tests using *E. coli* also support lack of genotoxic activity in bacterial systems (59, 60, 61, 62, 63). A chromosome aberration study in CHO cells, conducted for the National Toxicology Program (and prepared as a robust summary for this HPV document) gave a reproducible positive result in the presence of high concentration of test material and metabolic activation but not in the absence of the metabolic activation system (25). In a similar study investigating the induction of sister chromatid exchanges by γ-Butyrolactone, it was found that high concentrations (above 2,500 mcg/mL) of γ-Butyrolactone induced a significant increase of SCE's in

Chinese hamster ovary cells in the presence but not the absence of metabolic activation. In this report (64), both endpoints (the Loveday publication also covers the CHO chromosome aberration study) were significantly increased only in the presence of induced *S9* and the authors speculated that the addition of S9 enzymes coupled with 10-fold higher concentration of γ-Butyrolactone allowed detection of cytogenetic effects which were not observed in the earlier negative study with a rat liver cell line (65).

Tests in yeast for mitotic gene conversion and aneuploidy induction were also negative (66). Negative results were obtained with γ-Butyrolactone in tests for chromosome aberration induction using a rat liver epithelial cell line without supplemental *S9* (67) and in tests for unscheduled DNA repair in HeLa cells with and without S9 (68). γ-Butyrolactone was also negative for induction of gene mutations in Chinese hamster *V79* cells (69) and human fibroblasts (70) with and without S9.

Genetic Toxicology in vivo

Mammalian genotoxicity was assessed *in vivo* using the Mouse Micronucleus Test as reported by multiple investigators. One representative test result had been included in the robust summaries (71) and the others are supporting (72, 73, 74). In this study, two i.p. doses of γ-Butyrolactone given at 80% of the LD_{50} with intervals between dosing and sacrifice of 24, 48, 72 or 96 hours, failed to produce an increase in polychromatic erythrocytes containing micronuclei. It was concluded that the test material did not show genotoxic activity in this system (71). Additional negative *in vivo* studies have been reported in *Drosophila melanogaster* (75).

Recommendation: The SIDS requirement for genetic testing has been met as assays sensitive to both point mutation and to clastogenic effects have been conducted using acceptable protocols. No additional testing is recommended.

Reproductive Toxicity

Reproductive effects have been adequately assessed through the combination of the negative developmental toxicity study (82) and the subchronic (and chronic) studies in which reproductive organs were evaluated and found to be unaffected by treatment with γ-Butyrolactone (25). Conduct of additional studies would not add significantly to our understanding of this material's reproductive toxicity.

Potentially relevant to reproductive toxicity, one published study described the inhibition of ovulation in rats by γ-Butyrolactone (76). In this study, the investigators examined the effect of γ-Hydroxybutyrate when administered in proestrous on ovulation in rats. They had postulated that γ-Hydroxybutyrate, which had been reported to produce a significant increase in dopamine without affecting other brain neurotransmitters, might result in reduction in the production of FSH and/or LH by the pituitary. This reduction in FSH and LH surge has the potential to interfere with ovulation in the rat. Their results showed that after a single i.p. injection of γ-Butyrolactone in proestrus, serum LH and especially FSH levels were reduced, in a dose-dependent manner, from one to four hours after the injection. In addition, the number of rats ovulating was reduced in a dose dependent manner. The ED_{50} for antiovulatory activity in the Sprague-Dawley rat was determined to be approximately 250 mg/kg, which was below the anesthetic dose. This finding is biologically plausible as it is known that ovulation is under control of a neuroendocrine cascade that involves receptors in the hypothalamus that are sensitive to dopamine or GABA and that cause the release of GnRH into the pituitary with subsequent release of LH and FSH, which are required for ovulation.

The relevance of this finding in rats for humans is unknown. Some considerations are that humans have a menstrual cycle rather than an estrous cycle; however, the same neuroendocrinal substances are involved (77). Another factor is that rats quickly develop a tolerance to the sedative action of γ-Hydroxybutyrate (78) and it has been proposed that this is associated with a down-regulation of the γ-Hydroxybutyrate receptors (79). If it is also the case that the neuronal systems regulating GnRH are subject to the rapid development of tolerance, then repeated dosing of animals with γ-Butyrolactone might not affect ovulation. It is also controversial if and under what conditions of administration γ-Hydroxybutyrate elevates or lowers CNS dopamine levels (80, 81). These investigators have also presented evidence that the effect of γ-Hydroxybutyrate on CNS dopamine levels can be reversed by varying the route of administration from i.p. to s.c. injection, implicating the route of administration as a critical variable. In conclusion, not enough is known about the mechanisms involved to make an informed decision concerning the potential of γ-Butyrolactone to act as an ovulation inhibiter in humans. In addition, due to the significant differences in ovulatory cycles between humans and rodents, it is questionable if additional studies of reproductive toxicity in rodents that focus on antiovulatory effects will add any information of value to human hazard or risk assessment.

Recommendation: No additional reproductive testing is recommended, as the available data are sufficient to assess the reproductive toxicity of this material according to the HPV guidelines.

Developmental Toxicity

A modern OECD 414 Guideline study has been conducted with γ-Butyrolactone. The results of this investigation conducted in rabbits using inhalation at 0.5, 1.4 and 5.0 mg/L for 6 hours per day on days 9 to 17 of gestation did not produce either maternal or fetal toxicity. None of the reproductive, embryonic, or fetal parameters was affected by this treatment at the highest recommended inhalation dose. The high dose level also exceeded the saturation vapor concentration of γ-Butyrolactone in air and was conducted as a mixed vapor-aerosol exposure. Both the developmental and maternal NOAEL was 5.0 mg/L (82).

This result is supported by a study in which groups of 10 pregnant Sprague-Dawley-rats were given 0, 10, 50, 125, 250, or 500 mg/kg γ-Butyrolactone by gavage on days six through 15 of gestation (83). Dams were observed for signs of intoxication, body weights were measured daily from days zero through 21 of gestation, and food and water consumption were monitored at 3-day intervals. Dams were killed on day 21, and the uteri were removed. Fetal data were recorded and fetuses were examined for malformations. Placental weights were significantly reduced in treated animals at all doses. Mean fetal weights were significantly increased in the 50, 125, and 250-mg/kg groups. No other treatment related changes of significance were seen either in the dams or fetuses. The maximum dose was controlled by the solubility of the test substance in the vehicle and as there was no maternal toxicity produced, a higher dose-level might have been achieved with an alternate vehicle. This study indicates no developmental hazard up to 500-mg/kg body weight but does not define the maternal or developmental LOAEL. As this dose level is half of the OECD-recommended maximum of 1000 mg/kg in the current OECD 414 test and as no adverse effects were produced, this study supports low developmental hazard.

Taken together, the two rat developmental toxicity studies indicate a low developmental toxicity hazard for γ-Butyrolactone by the inhalation or oral routes

Recommendation: No additional developmental toxicity testing is required as the available data are sufficient to assess the developmental toxicity of this material.

Conclusions

With regard to the parameters specified in the EPA HPV Challenge program, it is concluded that the available information on 2-Pyrrolidone fills all of the requirements for physicochemical parameters, fate information, aquatic toxicity and mammalian toxicity. Although the available studies do not meet all the requirements of the current OECD guidelines in all cases, taken together the information provided a reliable hazard assessment. Conduct of additional studies would not add significantly to our understanding of this material's toxicity.

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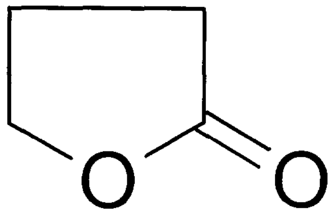
201-14221B

γ-Butyrolactone



CAS Number 96-48-0

Existing Chemical	:	ID: 96-48-0
CAS No.	:	96-48-0
EINECS Name	:	gamma-butyrolactone
EC No.	:	202-509-5
TSCA Name	:	2(3H)-Furanone, dihydro-
Molecular Formula	:	C4H6O2

Producer related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	13.10.2002

Substance related part

Company	:	Toxicology and Regulatory Affairs
Creation date	:	13.10.2002
Status	:	
Memo	:	
Printing date	:	31.12.2002
Revision date	:	
Date of last update	:	31.12.2002
Number of pages	:	42
Chapter (profile)	:	Chapter: 1.0.1, 1.2, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6.1, 3.1.1, 3.1.2, 3.3.1, 3.3.2, 3.5, 4.1, 4.2, 4.3, 4.4, 5.1.1, 5.1.2, 5.1.3, 5.1.4, 5.4, 5.5, 5.6, 5.7, 5.8.1, 5.8.2
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	Toxicology and Regulatory Affairs
Contact person	:	Elmer Rauckman PhD DABT
Date	:	
Street	:	1201 Anise Court
Town	:	62243-2118 Freeburg, IL
Country	:	United States
Phone	:	618-539-5280
Telefax	:	618-539-5394
Telex	:	
Cedex	:	
Email	:	rauckman@toxicsolutions.com
Homepage	:	toxicsolutions.com
Remark	:	Participating Members of Consortium BASF Corporation International Specialty Products

31.12.2002

1.2 SYNONYMS AND TRADENAMES

2.1 MELTING POINT

Value : = -43.5 °C

Remark : Supported by value in Merck Index, Thirteenth Ed, 2001
Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Handbook value
Flag : Critical study for SIDS endpoint
13.10.2002 (20)

2.2 BOILING POINT

Value : = 204 °C at 1013 hPa

Remark : Supported by IUCLID 2000 value of 204-206 deg C
Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Handbook value
Flag : Critical study for SIDS endpoint
13.10.2002 (23)

2.3 DENSITY

Type : relative density
Value : = 1.1284 at 16 °C

Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Handbook value
Flag : Critical study for SIDS endpoint
13.10.2002 (20)

2.4 VAPOUR PRESSURE

Value : = .344 hPa at 20 °C

Remark : Supported by handbook value of 0.60 hPa (0,45 mm Hg) @ 25 C in: Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989
Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
13.10.2002 (2)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = -.64 at 25 °C
pH value :

Test substance :
gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Handbook value
19.12.2002 (16)

Partition coefficient : octanol-water
Log pow : = -.566 at °C
pH value :
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 1987
GLP : no data
Test substance :

Remark : Depending on the duration of the study and the pH of the aqueous phase, the test may have measured the partition coefficient of a mixture of gamma-butyrolactone, gamma-hydroxybutyric acid and gamma-hydroxybutyrate. See water stability section for more explanation.
Reliability : (2) valid with restrictions
19.12.2002 (1)

Partition coefficient : octanol-water
Log pow : = .59 at 20 °C
pH value :
Method : OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year : 1999
GLP : yes
Test substance :

Remark : None of the callibration standards were lower in log Kow than test substance.

Test considered Invalid
Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (3) invalid
Invalid, test substance unsuitable for method employed
19.12.2002 (13)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	miscible
Stable	:	no
Deg. product	:	yes
Method	:	
Year	:	
GLP	:	
Test substance	:	
Test substance	:	gamma-butyrolactone, CASNO 96-48-0
Reliability	:	(2) valid with restrictions Handbook value
Flag	:	Critical study for SIDS endpoint

19.12.2002 (23)

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
DIRECT PHOTOLYSIS
Halflife t1/2 : ca. 56 hour(s)
Degradation : % after
Quantum yield :
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 1500000 molecule/cm^3
Rate constant : ca. .0000000000023 cm^3/(molecule*sec)
Degradation : % after
Deg. product :
Method :
Year : 2002
GLP :
Test substance :

Method : Calculated with AOP v1.90 Program based on SMILES structure
Result :

AOP Program (v1.90) Results:
==========================
SMILES : C1CCC(=O)O1
CHEM : BLO
MOL FOR: C4 H6 O2
MOL WT : 86.09
------- SUMMARY (AOP v1.90): HYDROXYL RADICALS ----------

Hydrogen Abstraction = 2.3087 E-12 cm3/molecule-sec
Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/molecule-sec
Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec

OVERALL OH Rate Constant = 2.3087 E-12 cm3/molecule-sec
HALF-LIFE = 4.633 Days (12-hr day; 1.5E6 OH/cm3)
HALF-LIFE = 55.596 Hrs

Source : Toxicology and Regulatory Affairs Calculation -2002
Test substance :
gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Calculated by an acceptable method
Flag : Critical study for SIDS endpoint
30.12.2002 (14)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : ca. 14 - 28 day(s) at 20 °C
t1/2 pH9 : at °C
t1/2 pH 12 : < 5 minute(s) at 20 °C
Deg. product :
Method :
Year :
GLP : no data
Test substance :

Method : Stock solutions of 1% w/w GHB or GBL were prepared in deionized water. Potassium phosphate monobasic solution (1 M) was prepared and then adjusted to pH 2.0, 4.0, 5.2, 6.4, 7.0, or 12.0 using aqueous phosphoric acid or sodium hydroxide. Preparation of GHB or GBL in the various buffers was done by mixing equal volumes (1 mL) stock solution and buffer in a 5 mL amber glass bottles that was vortexed for 10 s. For preparation of in deionized water, water was substituted for the buffer portion. The final GHB or GBL concentrations were 0.5% w/w in 0.5 M buffer or deionized water. All solutions were prepared in duplicate and stored under ambient conditions at 22°C without further mixing. "Time zero" measurements were made by analysis immediately after vortexing. The actual time between contact of the GHB or GBL solution with the buffer and injection for analysis is estimated as less than 2 min. Due to the rapid conversion of GBL to GHB at pH 12.0, it was necessary to quench the reaction by adding 1 mL of the pH 2.0 buffer after the specified reaction time. The pH after quenching the reaction was ca. 6.2.

The formation of GHB from GBL in solution was confirmed by conducting either or both GCMS or infrared analysis on the test solutions. The presence of GHB and GBL in the commercial and clandestine GBL products was also confirmed by conducting both GC-MS and infrared analysis.

Remark :

These results indicate that butyrolactone will be hydrolyzed readily under environmental aquatic conditions. The buffered pH 7 solution results suggest that its hydrolytic half-life in the environment at neutral pH is in the range of 2 to 4 weeks.

This result is supported by a textbook literature value cited as 72% GBL and 27% GHB (14) without specifying the exact conditions that produced the equilibrium mixture were or the primary reference. (Streitwieser Jr. A, Heathcock CH. Introduction to organic chemistry. New York: Macmillan Publishing Co., Inc., 1976.)

Result :

Under these conditions, hydrolysis of GBL in pure water proceeded slowly over a period of months, and reached stable ratio comprising ca. 2:1 GBL:GHB (67% GBL; 33% GHB) within 202 days. The solution pH decreased, reaching and maintaining a pH of ca. 3.3 after 108 days of storage. The decrease in pH was attributed to the partial dissociation of the GHB free acid upon forming. The results observed for the GBL-pure water solutions are consistent with the slow formation of an equilibrium mixture of

GHB and GBL.

At pH 2.0, the hydrolysis of GBL was much more rapid than in pure water, and produced a similar stable reaction mixture (68% GBL; 32% GHB) within only nine days of storage.

If the study was started with GHB at pH 2.0, the reaction mixture (67% GBL: 33% GHB) was again produced within 9 days of storage. The formation of the same stable reaction mixture starting from either GHB or GBL at pH 2.0 is evidence of a true equilibrium. The reaction mixture was monitored for 202 days and the composition remained constant.

The hydrolysis of GBL at pH 12.0 occurred rapidly, with greater than 90% conversion to GHB within 5 min, and complete conversion within 15 min. The current study, the reaction mixture was monitored for nearly seven months (202 days) and was stable.

In buffered solutions at pH 7.0, the hydrolysis of GBL proceeded more rapidly than in pure water and was also observed to proceed to near completion (97% conversion to GHB at the end of the study, 202 days). These results are predicted because the solution pH was maintained at 7.0 in the buffer, and nearly all of the GHB that formed ultimately dissociated to the anion or salt form (pKa of GHB estimated about 5.0, driving the reaction to near completion. For pH 4.0, 5.2, and 6.4 buffered solutions, both the rate and extent of GBL hydrolysis were lower than at pH 7.0.

Test substance :
gamma-butyrolactone, CASNO 96-48-0, From Spectrum Chemical, purity > 98%

Conclusion :
gamma-butyrolactone will be hydrolyzed readily under environmental aquatic conditions.

Reliability : (1) valid without restriction
Acceptable published study with sufficient detail.

Flag : Critical study for SIDS endpoint

30.12.2002 (11)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year : 2002

Method : EQC Level 3 calculation using EPIWIN 3.05 with measured values of physical parameters and biodegredation times adjusted for available data through user input. See results for values employed

Result :

Level III Fugacity Model (Full-Output):
===========================
Chem Name : BLO
Molecular Wt: 86.09
Henry's LC : 5.27e-008 atm-m3/mole (Henry database)
Vapor Press : 0.259 mm Hg (user-entered)

Log Kow : -0.64 (user-entered)
Soil Koc : 0.0939 (calc by model)

	Concentration (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	3.21	111	1000
Water	34.3	100	1000
Soil	62.4	150	1000
Sediment	0.0182	100	0

	Fugacity (atm)	Reaction (kg/hr)	Advection (kg/hr)	Reaction (percent)	Advection (percent)
Air	4.46e-011	98.2	157	3.27	5.24
Water	5.14e-013	1.16e+003	168	38.8	5.6
Soil	3.44e-011	1.41e+003	0	47	0
Sedmt	1.36e-013	0.617	0.0018	0.0206	5.93e-005

Persistence Time: 163 hr
Reaction Time: 183 hr
Advection Time: 1.5e+003 hr
Percent Reacted: 89.2
Percent Advected: 10.8

Half-Lives (hr), (based upon user-entry):
Air: 111
Water: 100
Soil: 150
Sediment: 100

Advection Times (hr):
Air: 100
Water: 1000
Sediment: 5e+004

Source : Calculation by Toxicology and Regulatory Affairs
Test substance :

gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions

Calculated by an acceptable method
Flag : Critical study for SIDS endpoint
30.12.2002 (15)

3.5 BIODEGRADATION

Type : aerobic
Inoculum :
Contact time :
Degradation : = 60 - 92 (±) % after 14 day(s)
Result : readily biodegradable
Deg. product :
Method : other: MITI Test
Year :
GLP :
Test substance :

Method : MITI Test

Result :
Using the MITI test, a biodegredation of 60-92% was observed afer 14 days

Test substance :
gamma-butyrolactone, CASNO 96-48-0

Conclusion :
Readily Biodegradable

Reliability : (2) valid with restrictions
Acceptable publication

Flag : Critical study for SIDS endpoint

30.12.2002 (10)

Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 2 mg/l related to Test substance
3 mg/l related to Test substance
Contact time :
Degradation : >= 95 (±) % after 8 day(s)
Result : inherently biodegradable

Method : In this BOD test, 2.0, 3.0 or 4.5 mg/L test material was incubated in a volume of 3000 ml water with essential salts and 150 ml non-adapted sludge using triplicate flasks. Oxygen determinations were conducted at 3 hours and at 1, 5, 8, 12, and 13 days of incubation. TOC determinations were conducted at 3 hours and at 12, and 13 days of incubation.

Result :
The oxygen demand for the high concentration exceeded the available oxygen and the data were not used. The oxygen demand in the two lower concentrations was 3.65 mg/L at 2 mg/L and 5.9 mg/L at 3.0 mg/L test substance. These levels were attained by day-5 of incubation. These oxygen demand levels correspond to 100% elimination.

The TOC levels indicated 95, 96 and 99% degradation at 5, 12 and 13 days of incubation, respectively.

Test substance :
gamma-butyrolactone, CASNO 96-48-0

Conclusion :
Test material biodegrades rapidly using a non-acclimated inoculate of activated sludge.

30.12.2002 (5)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	=
LC50	:	= 275 - 302
LC100	:	= 302
Limit test	:	no
Analytical monitoring	:	no data
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method : After several preliminary tests for rangefinding, 10 fish (golden orfe, mean wt 1.94 g) were exposed to the test material at several closely spaced concentrations. Conditions, including water parameters, volume of containers, lighting or temperature were not specified on the data sheet. pH and oxygen levels were determined in representative preliminary tests.

Remark :

Supporting this result, the toxicity was modeled using the EPA developed ECOSAR program (ver 0.99f) run with the "esters" model and the measured Ko/w of -0.64.

This model predicts a 96-hr LC50 of 334 mg/L, in good agreement with the experimental value.

Result :

The preliminary test data are not included here. In the definitive test, the following results were recorded

Conc (mg/L)	# fish	#dead at 4 hr	24 hr	48 hr
250	10	0	0	0
275	10	0	1	1
302	10	0	10	10
331	10	0	10	10
364	10	0	10	10
400	10	0	10	10
0	10	0	0	0

The pH value in the 400 mg/L concentration (preliminary test with 5 fish) was initially 7.0 and was 6.7 at the end (48 hours) of the study

Oxygen levels were not determined at study termination.

Test substance :

gamma-butyrolactone, CASNO 96-48-0

Conclusion : The LC50 for the Golden orfe under these conditions is between 275 and 302/ mg/L.

Reliability : (2) valid with restrictions
Although many details of the test were not recorded, it was conducted by the standard procedure of the time and the original data sheets were available for review.

Flag : Critical study for SIDS endpoint

30.12.2002 (6)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	= 500
EC50	:	> 500
Limit Test	:	no
Analytical monitoring	:	no
Method	:	Directive 84/449/EEC, C.2 "Acute toxicity for Daphnia"
Year	:	
GLP	:	no data
Test substance	:	

Method : This study was conducted in accordance with Directive 84/449/EEC

Groups of 20 daphnids (4 replicates of 5 animals) were exposed to four nominal concentrations of test substance for a period of 48 hours. Animals (2 to 24 hours old) were examined for immobilization at 0, 3, 6, 24, and 48 hours after starting the exposure.

Remark :

Two issues are potential confounders in this study. The first is volatility; however, based on the vapor pressure and water solubility (Henry's Law constant) of the test material, this is considered to not be an issue.

The second issue is water stability of the test material. Data have shown that this material converts to gamma-hydroxybutyrate in neutral and basic solution. The kinetics of this hydrolysis are well established and at pH 7 it is known there is only about a 15% hydrolysis in 3 days. Based on the known pH dependency at it can be extrapolated that the 48 hour loss at pH 8 would be less than 20%. In addition, there is a supporting study from the same laboratory extending the LC 50 to > 1000 mg/L. This is not a definitive study; however, as only two replicates of 5 daphnids each (10 daphnids per concentration) were utilized under conditions identical to the test using 20 (BASF AG, unpublished results 18 December 1988).

Supporting this result, the toxicity was modeled using the EPA developed ECOSAR program (ver 0.99f) run with the "esters" model and the measured Ko/w of -0.64.

This model predicts a 96-hr EC50 of 17300 mg/L, in agreement with the experimental value.

Result :

No animal died at any of the test concentrations of 0, 62.5, 125, 250, or 500 mg/L. Initial pH was 8.2-8.3, final pH was 7.52 to 7.98 with lower values at higher concentrations. Temperature was 292° K. TOC was not reported. Oxygen concentration was measures in a parallel set of vessels and was above 6.5 mg/L in all concentrations at the beginning and end of the study.

Test condition :

Vessels were glass centrifuge tubes containing 10 ml of test solution. The dilution water was filtered tap-water with the chlorine removed by passing the water over activated carbon and had a hardness of 2.7 mmol/L, an alkalinity of 0.80 mmol/L and Ratios of Ca: Mg of 4:1 and Na:K of 10:1. Lighting was diffuse 550-650 microSiemons/cm on a 16-hour light, 8-hour dark cycle. Initial pH was 7.7-8.3.

Test substance : gamma-butyrolactone, CASNO 96-48-0, purity > 99.5%
Conclusion : The LC50 is > 500 mg/L
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.12.2002 (3)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Scenedesmus subspicatus (Algae)
Endpoint : biomass
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : = 78.7 measured/nominal
EC20 : = 20.1 measured/nominal
Limit test : no
Analytical monitoring :
Method :
Year :
GLP : no data
Test substance :

Method : Cells were inoculated at 10000 cell/ml into replicate (quadruplicate) 250 Erlenmeyer flasks containing 100 ml of test material in algae growth medium (OECD). Cultures were incubated at 293 ° K for 96 hours under 6.2 miS/cm lighting. Cell growth was measured fluorometrically in all flasks at 24, 48, 72 and 96 hours of incubation.

Two individual experiments were conducted, as the first did not define an IC20.

Remark :

Two issues are potential confounders in this study. The first is volatility; however, based on the vapor pressure and water solubility (Henry's Law Constant) of the test material, this is considered to not be an issue.

The second issue is water stability of the test material. Data have shown that this material converts to gamma-hydroxybutyrate in neutral and basic solution. The kinetics of this hydrolysis are well established and at pH 7 it is known there is only about a 15% hydrolysis in 3 days. Based on the known pH dependency at it can be extrapolated that the 96-hour loss at pH 8 would be less than 30%. The lower concentration inoculated flasks, where the pH exceeded 10 at the 96-hour interval, probably contained at least 50% of the test material as gamma-hydroxybutyrate.

Supporting this result, the toxicity was modeled using the EPA developed ECOSAR program (ver 0.99f) run with the "esters" model and the measured Ko/w of -0.64.

This model predicts a 96-hr EC50 of 24 mg/L, in good agreement with the experimental value.

Result :

In the first study test solutions were 0, 31.25, 62.5, 125, 250 or 500 mg/L of test substance. The 96-hr IC50 from this study was 89 mg/L.

In the second study test solutions were 0, 7.8, 15.6, 31.3, 62.5, 125, 250 or 500 mg/L of test substance. The percent growth as compared to control at 96 hours was 92, 85, 67, 53, 41, 34 and 27%, low to high concentration, respectively. The 96-hour IC50 was determined to be 79 mg/L and the 96-hour IC20 was 20.1 mg/L. IC50 and IC20 for other times were: 72-hour, 359 and 14.3 mg/L; 48-hour, > 500 and 37.1 mg/L; 24-hour, >500 and > 500.

pH values at 0 hours for non-inoculated flasks from control to high concentrations were: 8.15, 8.12, 8.11, 8.10, 8.10, 8.06, 8.01, 7.92

pH values at 0 hours for inoculated flasks from control to high concentrations were: 8.12, 8.15, 8.14, 8.13, 8.12, 8.09, 8.05, 7.99

pH values at 72 hours for non-inoculated flasks from control to high concentrations were: 8.08, 8.08, 8.08, 8.06, 7.99, 7.91, 7.73, 7.51

pH values at 72 hours for inoculated flasks from control to high concentrations were: 10.16, 10.13, 10.11, 9.92, 9.33, 8.54, 7.78, 7.36

Test substance : gamma-butyrolactone, CASNO 96-48-0

Reliability : (1) valid without restriction

Guideline-like study with confirmatory study.

Flag : Critical study for SIDS endpoint

30.12.2002 (7)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 1920 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : water
Doses : 0.5, 0.75, 1.0, 1.25, 2.0, 3.0, 4.0 cc/kg
Method :
Year :
GLP : no
Test substance :

Method : Groups of 10 white rats were given test substance (10% v/v in water) by gavage after a 2-week acclimatizing period. The dose levels were 5, 4, 3, 2, 1.25, 1.0, 0.75, 0.5 cc/kg. Animals were observed for 2 weeks after dosing. All animals that died were necropsied (gross pathology only).

Remark :

This result supported by an LD50 of 1920 mg/kg found for guinea pigs using an almost identical protocol reported in the same laboratory report.

Result :

The results were:

5 cc/kg: All rats died within 24 hours.
4 cc/kg: All rats died within 24 hours.
3 cc/kg: 6 died within 24 h, 2 died from 24-48 h, 2 died from 48-72 h
2 cc/kg: 2 survived 14 days, 4 died within 24 h, 2 died from 24-48 h, 2 died from 48-72 h
1.25 cc/kg: 5 survived 14 days, 3 died from 24-48 h, 2 died from 48-72 h
1.0 cc/kg: 8 survived 14 days, 2 died from 48-72 h
0.75 cc/kg: 10 survived 14-day observation period
0.50 cc/kg: 10 survived 14-day observation period

Test substance :

gamma-butyrolactone, CASNO 96-48-0

Conclusion :

LD-0 = 0.75 cc/kg
LD-50 = 1.5 cc/kg
LD-100 = 3 cc/kg

Based on a density of 1.28 g/cc these are

LD-0 = 960 mg/kg
LD-50 = 1920 mg/kg
LD-100 = 3840 mg/kg

Reliability : (2) valid with restrictions
Although some details missing, study was conducted using a scientifically defensible method. Orignial laboratory reprort available.

Flag : Critical study for SIDS endpoint
30.12.2002 (18)

Type : LD50
Value : = 1580 mg/kg bw
Species : rat

Strain : no data
Sex : no data
Number of animals :
Vehicle : water
Doses : not specified
Method :
Year :
GLP : no
Test substance :

Method : A 7-day LD50 determination was conducted by oral dosing of rats with test material in aqueous solution. Dose levels, group size, and mortality by dose are not specified.
Result :
Within a few minutes of administration, rats acted intoxicated, remained on their stomach or side, or were comatose. Animals that survived appeared normal within 24 hours. Those that died did so within 24 hours. No other details reported.
Test substance :
gamma-butyrolactone, CASNO 96-48-0
Conclusion :
Within a few minutes of administration, rats acted intoxicated, remained on their stomach or side, or were comatose. Animals that survived appeared normal within 24 hours. Those that died did so within 24 hours. No chemically-related organ findings were made on necropsy. No other details reported.
Reliability : (2) valid with restrictions
30.12.2002 (8)

5.1.2 ACUTE INHALATION TOXICITY

Type : other: Limit test
Value : > 300 ppm
Species : rat
Strain : no data
Sex : no data
Number of animals : 6
Vehicle : other: air
Doses : Saturation
Exposure time : 8 hour(s)
Method :
Year :
GLP : no
Test substance :

Method : A group of six rats were exposed to air that was saturated with gamma-butyrolactone vapor at 20 deg C for a period of eight hours. Animals were observed for 7 days after the exposure.
Remark :
At saturation, based on the reported vapor pressure of 0.344 hPa, air would contain ca. 339 ppm vapor.

This is ca 1200 mg/M3 or 1.2 mg/L.

No other details of this study were provided in the report.

Result	:	All rats survived and they were described as being "symptom free" for the entire observation period.
Test substance	:	gamma-butyrolactone, CASNO 96-48-0
Conclusion	:	The 8-hour Inhalation LD50 is greater than saturation at 20 ged C
Reliability	:	(2) valid with restrictions Conducted by a scientifically defensible method

30.12.2002 (8)

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	5 days a week
Post exposure period	:	
Doses	:	56, 112, 225, 450, or 900 mg/kg of body weight
Control group	:	yes, concurrent vehicle
NOAEL	:	= 225 - 450 ml/kg bw
LOAEL	:	= 450 - 900 ml/kg bw
Method	:	other: NTP SOW
Year	:	
GLP	:	yes
Test substance	:	

Method : Male and female F344/N rats, obtained from Charles River Breeding Laboratories (Kingston, NY), were observed for 19 days before the study started. The average age of rats was 51 days old at the beginning of the study. Groups of 10 rats received test material by gavage at doses of 0, 56, 112, 225, 450, or 900 mg/kg of body weight 5 days a week for 13 weeks. Water and feed were available ad libitum. Animals were observed twice a day and clinical observations were recorded once a week. Animals were weighed at the start of the study and weekly thereafter. Rats were housed five to a solid-bottom polycarbonate cage and the light cycle was 12-hour light and dark. Temperature was maintained between 22-24 deg C with RH of 35-62%. Surviving animals were killed at the end of the 13-week studies. Necropsies were performed on all study animals. The brain, heart, right kidney, liver, lungs, and thymus of survivors were weighed at necropsy. Complete histopathology was performed on all animals killed or dying during the study, all control animals, rats receiving 900 mg/kg, male rats receiving 450 mg/kg. The liver and nose (nasal cavity and turbinates) were examined from rats in the 56, 112, and 225 mg/kg dose groups and from female rats in the 450 mg/kg dose groups. Tissues routinely examined include: adrenal gland, bone and marrow (femur), brain, clitoral gland, esophagus, epididymis, heart, kidney, large intestine, liver, lung with mainstem bronchi, lymph nodes (mesenteric, mandibular), mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes).

Result :

All high-dose males and one high-dose female died. Males receiving 450 mg/kg gained less body weight. There was no body-weight effect in females at any dose level. Other than inflammation of the nasal mucosa in all groups of dosed rats, there were no specific organ effects. The nasal mucosa irritation was considered to be a non-specific effect of gavage with a volatile agent.

Rats at the higher dose levels (225 mg/kg and above) showed signs of sedation after dosing during the first 2-3 weeks of study that diminished in intensity with continued dosing, and rats showed no visible signs of sedation after three weeks of dosing.

Test substance :

gamma-butyrolactone, CASNO 96-48-0, purity > 97%

Conclusion :

The NOAEL was 225 mg/kg for males (based on body weights), and 450 mg/kg for females (based on one death in the 900-mg/kg group). No specific target organs were identified.

Reliability : (1) valid without restriction
Guideline study under glp with no deviations

Flag : Critical study for SIDS endpoint

30.12.2002 (21)

Type : Sub-chronic
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 13 weeks
Frequency of treatm. : 5 days a week
Post exposure period :
Doses : 65, 131, 262, 525, or 1,050 mg/kg
Control group : yes, concurrent vehicle
NOAEL : = 525 mg/kg bw
LOAEL : = 1050 ml/kg bw
Method : other: NTP SOW
Year :
GLP :
Test substance :

Method : B6C3F1 mice of each sex obtained from Charles River Breeding Laboratories (Kingston, NY) were observed for 19 days before the start of dosing. The average age of the mice was 58 days old at the first dosing. Groups of 10 mice received test material by corn-oil gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg 5 days a week for 13 weeks. Water and feed were available ad libitum. Animals were housed five to a solid-bottom polycarbonate cage and the light cycle was 12-hour light and dark. Temperature was maintained between 22-24 deg C with RH of 35-62%. Animals were observed twice a day and clinical observations were recorded once weekly. Animals were weighed at the start, of the study and weekly thereafter. Surviving animals were killed at the end of the 13-week studies. Necropsies were performed on all study animals. The brain, heart, right kidney, liver, lungs, and thymus of survivors were weighed at necropsy. Complete histopathology was performed on all animals killed or dying during the study, all control animals, and mice receiving 1,050 mg/kg. Tissues examined included: adrenal gland, bone and marrow (femur), brain, preputial gland, esophagus, gallbladder, heart, kidney, large intestine, liver, lung with mainstem bronchi, lymph nodes (mesenteric, mandibular), mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes).

Result :

Three high-dose males and one high-dose female died as a result of exposure. High-dose males gained less body weight than controls. There were no gross or microscopic lesions. Mice at the two highest dose levels showed signs of sedation after dosing during the first 2-3 weeks of study that diminished in intensity with continued dosing.

Test substance :

gamma-butyrolactone, CASNO 96-48-0, purity > 97%

Conclusion :

Except for minor sedation during the first few weeks of study, the NOAEL was 525 mg/kg for males (based on body weights and mortality), and 525 mg/kg for females (based on one death in the 1050-mg/kg group). No specific target organs were identified.

Reliability : (1) valid without restriction
Guideline study under glp with no deviations

Flag : Critical study for SIDS endpoint

30.12.2002 (21)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay
System of testing :
Test concentration : 0, 33, 100, 333, 1000, 3333, 10000 mcg/plqte
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method :
Year :
GLP : no data
Test substance :

Method : Liver S-9 fractions were prepared from male Sprague-Dawley rats and male Syrian hamsters that were induced with Aroclor 1254 (200 mg/ml in corn oil) at 500 mg/kg. Five days after injection, animals were sacrificed by decapitation and the livers removed aseptically. Animals were fasted for 12-24 hr immediately preceding sacrifice. Liver homogenates were prepared aseptically at 0-4°C. Excised livers were rinsed with 0.15 M KCl, then minced and homogenized (3 ml of 0.15 M KCl/g wet tissue) in a Potter-Elvehjem apparatus with a teflon pestle. The homogenate was centrifuged for 10 min at 9,000g at 4°C. The supernatant (S-9) was decanted and distributed into freezing ampules and stored at -70°C. The microsomal enzyme reaction mix (S-9 mix) was prepared immediately prior to each assay. One milliliter of S-9 mix has the following composition: S-9, 0.10 ml; 0.04 M MgC12, 0.02 ml; 1.65 M KCl, 0.02 ml; 0.04 M ß-nicotinamide adenine dinucleotide phosphate (NADP), 0.10 ml; 0.05 M glucose-6-phosphate, 0.10 ml; 1.0 M NaH2PO4 (pH 7.4), 0.10 ml; and distilled water, 0.56 ml.

Preincubation Methodology::Material was tested using the preincubation procedure of the Salmonella assay. Briefly, 0.5 ml of S-9 mix or 0.1 M P04 buffer was dispensed into an appropriate number of 13 x 100 mm culture tubes maintained at 37°C in a dry-bath. Then, 0.05 ml of cells and 0.05 ml of solvent or chemical dilution were added to each tube. The mixture was vortexed and allowed to stand for 20 min at 37°C. Following the preincubation period, 2.0 ml molten top agar (45°C) supplemented with 0.5 mM L-histidine and 0.5 mM d-biotin was pipetted into the tubes, which were immediately vortexed, and their contents poured onto 25 ml of minimal glucose bottom agar in a 15 x 100-mm plastic petri dish (Falcon Muta-Assay, 1028). After the overlay solidified, the plates were inverted and incubated at 37°C for 48 h. The plates were then counted for (revertant) colonies, the results of theree paltes were averaged and reported.

At least five doses of test chemical, in addition to the concurrent solvent and positive controls, were tested on each strain in the presence of S-9 mix or buffer. Three plates were used, and the experiment was repeated no less than 1 week after completion of the initial test. To select the dose range for the mutagenesis assay, the test chemicals were checked for toxicity to TA100 up to a concentration of 10 mg/plate or the limit of solubility, both in the presence and absence of S-9 mix. One or more parameters were used as an indication of toxicity: viability on complete medium and reduced numbers of revertant colonies per plate and/or thinning or absence of the bacterial lawn. If toxicity was not apparent in the preliminary toxicity determination, the highest dose tested was 10 mg/plate; otherwise the upper limit of solubility was used. If toxicity was observed, the doses of test chemical were chosen so that the high dose exhibited some degree of toxicity.

Positive Controls:: The positive control chemicals were tested concurrently with each test chemical. 2-Aminoanthracene (2-AA) was tested on all strains in the presence of rat and hamster S-9. 4-Nitro-o-phenylenediamine (NOPD) was tested on TA98 without S-9. Also without S-9, sodium azide (SA) was tested on TA100 and TA1535, and 9-aminoacridine (9-AAD) was tested on TA1537. The actual concentration for each positive control chemical used for each strain and activation condition was selected based on dose-response curves generated at the beginning of the testing program. The doses of the positive controls are given in the results section.

Data Evaluation
The data were evaluated in an ad hoc manner by the testing laboratory (SRI International) and by NTP personnel. Prior to statistical analysis no formal rules were used; however, a positive response was indicated by a reproducible, dose-related increase, whether it be twofold over background or not. The matrix of test strains and activation systems used allowed the investigators to detect trends or patterns that might not be as evident if only one strain and activation system were examined. In addition to the standard "positive" and "negative" categories, there is also "questionable" (or "inconclusive"). This applied to low-level responses that were not reproducible within the laboratory or to results that showed a definite trend but with which the investigator did not feel comfortable in making a "+" or "-

" decision. It also included tests in which an elevated revertant colony yield occurred at only a single dose level. After a decision on the mutagenicity of a sample was made, a request to decode the sample was sent to the repository, and the code was broken. The data were subsequently evaluated using an analysis based on the models presented by Margolin et al [1981]. As a result of these statistical analyses, a number of calls on other test substances were changed from the original "negative" to "equivocal." The statistical analysis did not result in any "positive" or "equivocal" calls being called "negative." There was no change for this test substance.

Remark :

This Result is supported by the following published negative Salmonella Reverse Mutation Tests and other bacterial gene-mutation tests in E. coli (some results were uninterpretable but none were positive)

Baker, R.S.U., Bonin, A.M. Study of 42 coded compounds with the Salmonella/mammalian microsome assay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 249-260 (1981)

Garner, R.C., Welch, A., Pickering, C. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 280-284 (1981

Bridges, B.A., MacGregor, D., Zeiger, E. Summary report on the performance of bacterial mutation assays in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 49-67 (1981)

Ichinotsubo, D., Mower, H., Mandel, M. Mutagen testing of a series of paired compounds with the Ames Salmonella testing system in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 298-301 (1981)

Richold, M., Jones, E. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assayin: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 314-322 (1981)

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Simmon, V.F., Shepherd, G.F. Mutagenic activity of 42 coded compounds in the Salmonella/microsomeassay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 333-342 (1981)

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Venitt, S., Crofton-Sleigh, C. Mutagenicity of 42 coded compounds in a bacterial assay using Escherichia coli and Salmonella typhimuriumin: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 351-360 (1981)

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Martire. G., Vricella, G., Perfumo, A.M., De Lorenzo, F. Evaluation of the mutagenic activity of coded compounds in the Salmonella test in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 271-279 (1981)

Haworth, S., Lawlor, T., Mortelmans, K., Speck, W., Zeiger, E. Salmonella mutagenicity test results for 250 chemicals Environ. Mutagen., Suppl. 1, 3-142 (1983)

Nagao, M., Takahashi, Y. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 302-313 (1981)

Brooks, T.M., Dean, B.J. Mutagenic activity of 42 coded compounds in the Salmonella/microsome assay with Preincubation in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 261-270 (1981)

Aeschbacher, H.U., Wolleb, U., Lbliger, J., Spadone, J.C., Liardon, R. Contribution of coffee aroma constituents to the mutagenicity of coffee Food Chem. Toxicol., 27, 227-232 (1989)

Hubbard, S.A., Green, M.H.L., Bridges, B.A., Wain, A.J., Bridges, J.W. Fluctuation test with S9 and hepatocyte activation in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 361-370 (1981)

Gatehouse, D. Mutagenic activity of 42 coded compounds in the ‚microtiter" fluctuation test in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 376-386 (1981)

E.coli tests

Skopek, T.R., Andon, B.M., Kaden, D.A., Thilly, W.G. Mutagenic activity of 42 coded compounds using 8-azaguanine resistance as a marker genetic in Salmonella typhimurium in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 371-375 (1981

Matsushima, T., Takamoto, Y., Shirai, A., Sawamura, M., Sugimura, T. Reserve mutation test on 42 coded compounds with the E. coli WP2 system in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 387-395 (1981)

Kuroda, M., Yoshida, D., Mizusaki, S. Bio-antimutagenic effect of lactones on chemical mutagenesis in Escherichia coli Agric. Biol. Chem., 50, 243-245 (1986)

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DNA Repair Tests in E coil

Tweats, D.J. Activity of 42 coded compounds in a differential killing test using Escherichia coli strains WP2, WP67 (uvrA polA), and CM871 (uvrA lexA recA) in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 199-209 (1981)

Rosenkranz, H.S., Hyman, J., Leifer, Z. DNA polymerase deficient assay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 210-218 (1981)

Kada, T. The DNA-damaging activity of 42 coded compounds in the rec-assay in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 176-182 (1981)

Ichinotsubo, D., Mower, H., Mandel, M. Testing of a series of paired compounds (carcinogen and noncarcinogenic structural analog) by DNA repair-deficient E. coli strains in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 195-198 (1981 b)

Green, M.H.L. A differential killing test using an improved repair-deficient strain of Escherichia coli in: Progress in Mutation Research, Volume 1, Evaluation of Short-Term Tests for Carcinogens: Report of the International Collaborative Program De Serres FJ., Ashby J. Prog Mutat Res 1: 183-194 (1981)

Result :

Results from two replicate experiments were as follows:

EXPERIMENT 1

TA100

Dose	No S9	Rat S9	Hamster S9
0	120	116	143
100	125	129	142
333	125	130	143
1000	112	114	147
3333	123	122	136
10000	109	118	137
POS	227	688	1100

TA1535

Dose	No S9	Rat S9	Hamster S9
0	28	19	12
100	17	16	11
333	24	15	8
1000	23	12	8
3333	24	9	12
10000	28	16	10
POS	315	260	357

TA1537

Dose	No S9	Rat S9	Hamster S9
0	6	16	7
100	3	18	3
333	6	11	5
1000	5	9	8
3333	4	14	6
10000	4	12	7
POS	110	217	446

TA98

Dose	No S9	Rat S9	Hamster S9
0	18	24	29
100	21	25	33
333	17	29	31
1000	17	31	29
3333	16	29	35
10000	21	29	28
POS	654	462	926

#######EXPERIMENT 2 ##############

TA100

Dose	No S9	Rat S9	Hamster S9
0	105	118	121
100	109	134	115
333	115	136	122
1000	125	140	117
3333	116	111	119
10000	108	121	120
POS	419	495	778

TA1535

Dose	No S9	Rat S9	Hamster S9
0	24	11	8
100	28	15	11
333	23	20	9
1000	27	18	6
3333	24	20	11
10000	29	23	17
POS	379	120	356

TA1537

Dose	No S9	Rat S9	Hamster S9
0	8	16	6
100	8	13	9
333	7	11	6
1000	10	13	11
3333	8	13	12
10000	12	16	12
POS	277	204	454

TA98

Dose	No S9	Rat S9	Hamster S9
0	15	32	27
100	22	36	26
333	17	33	27
1000	17	33	27
3333	22	34	32
10000	15	37	28
POS	730	401	477

Test substance :
gamma-butyrolactone, CASNO 96-48-0, purity > 99.5%

Conclusion :
No mutagenic activity in this assay under these conditions

Reliability : (1) valid without restriction
Acceptable published study with sufficient detail.

Flag : Critical study for SIDS endpoint

30.12.2002 (17)

Type : Chromosomal aberration test
System of testing : CHO Cells in vitro
Test concentration : 400 to 4990 mcg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method :
Year :
GLP : no data
Test substance :

Method : Testing was performed as follows: Substance material was tested in cultured Chinese hamster ovary (CHO) cells for induction of chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridinesubstituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of y-butyrolactone; the high dose was limited to 5 mg/mL. Cells were incubated in McCoy's 5A medium with butyrolactone for 8 hours and Colcemid was added and incubated for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with butyrolactone and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. 100 first-division metaphase cells were scored at each dose. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. Abs data are presented as percentages of cells with aberrations. Both the dose-response curve and individual dose points were statistically analyzed.

Result :

Concentrations of of 2,580 to 3,990 mcg/ml butyrolactone caused significant increases in aberrations, with no evidence of cell cycle delay.

Trial 1 - No S9 Harvest time. 10.5 hours
Summary: Negative

		Cells	Abs	Ab/cell	%Cells with Abs
Medium					
		100	2	0.02	2.0
Mitomycin-C mcg/ml					
	5	100	31	0.31	22.0*
Butyrolactone mcg/ml					
	500	100	3	0.03	3.0
	1,500	100	2	0.02	2.0
	4,990	100	2	0.02	2.0

P=0.559

Trial 1 Plus S9 - Harvest time. 12.0 hours
Summary: Positive

		Cells	Abs	A/cell	%Cells with Abs
Medium					
		100	1	0.01	1.0
Cyclophosphamide mcg/ml					
	50	100	79	0.79	41.0*
Butyrolactone mcg/ml					
	400	100	0	0.00	0.0
	1,200	100	0	0.00	0.0
	1,500	100	2	0.02	2.0
	2,990	100	84	0.84	61.0*
	3,990	93	87	0.94	78.0*

P<0.001

Trial 2 - Plus S9 Harvest time: 12.0 hours

Summary: Positive		Cells	Abs	A/cell	%Cells with Abs
Medium					
		100	0	0.00	0.0
Cyclophosphamide mcg/ml					
	50	100	58	0.58	37.0
Butyrolactone mcg/ml					
	2,210	100	4	0.04	3.0
	2,580	100	7	0.07	7.04
	2,950	100	83	0.83	58.0*

P<0.001

Test substance : gamma-butyrolactone, CASNO 96-48-0

Reliability : (1) valid without restriction
Acceptable published study with sufficient detail.

30.12.2002 (22)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : mouse
Sex :
Strain : B6C3F1
Route of admin. : i.p.
Exposure period : 24, 48 or 72 hours after last treatment
Doses : 2x based on 80% of LD50
Result : negative
Method :
Year :
GLP : no data
Test substance :

Method : B6C3FI hybrid mice were purchased from Biobreeders Laboratory, Ottawa, Ontario. Test material was made up fresh in the appropriate solvent and administered intraperitoneally.

This investigation used a new protocol that incorporates multiple samples and consists of two phases In the first phase, mice were injected intraperitoneally with test agent at 0 and 24 hr, and samples were taken at 48, 72, and 96 hr. Each treatment consisted of a dose equal to 80% of the 7-day LD50. If there was a significant increase in the frequency of micronuclei at any sample time, then the treatment was repeated and animals sampled at the appropriate time or a graded series of doses were tested at the appropriate sample time. In either case, the agent was classified as clastogenic if there was a confirmation of the initial positive response, no further testing was performed. If in phase 1 or in the confirmation test no increase in the micronucleus frequency was detected, then a single treatment of either 50% or at both 80% and 40% of the 7-day LD50 was given and samples were taken at 30, 48, and 72 hr (phase 2). Where the response was negative for both phases, the agent was classified as nonclastogenic. However, when an increase in the frequency of micronuclei was noted in phase 2, a confirmation test was then performed. In general, when the results from phases 1 and 2 did not agree, a third test was used to reach a decision

The 7-day LD50 was determined to be 0.875 ml/kg in Male mice 30 weeks old weighing 30 g. (Same Journal issue page 684)

Result :

For this material Phase 1 and Phase 2 were negative giving the following number of micronuclei per 500 PCE at the indicated sampling times after 2 doses of 80% of the 7-day LD50:

Time	# PCE/Mouse (5 per group)
48 hours	0,0,0,1,1
72 hours	0,0,0,1,1
96 hours	0,0,0,1,0

Phase 2: 1 dose at 100% of the 7-day LD50

Time	# PCE/Mouse (5 per group)
36 hours	0,0,0,0,0
48 hours	0,0,0,0,0
72 hours	0,0,0,1,0

Phase 2: 1 dose at 50% of the 7-day LD50

Time	# PCE/Mouse (4 per group)
36 hours	0,0,0,0
48 hours	0,0,0,0
72 hours	0,0,0,0

Conclusion: Negative

Controls: Not shown. This was part of a study of the protocol on 41 coded compounds, many of which were known clastogens and were identified as positive by this protocol.

Test substance :

gamma-butyrolactone, CASNO 96-48-0

Reliability : (2) valid with restrictions
Acceptable published study with sufficient detail.

Flag : Critical study for SIDS endpoint

30.12.2002 (24)

5.7 CARCINOGENICITY

Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : gavage
Exposure period : 104 weeks
Frequency of treatm. : 5 days a week
Post exposure period :
Doses : Males 112 and 225 mg/kg-day; females 225 and 450 mg/kg-day
Result : negative
Control group : yes, concurrent vehicle
Method : other: NTP SOW
Year :
GLP : yes
Test substance :

Method : Groups of 50 rats of each sex were administered gamma-butyrolactone in corn oil by gavage 5 days a week for up to 103 weeks. Male rats received 0, 112, or 225 mg/kg, female received 0, 225, or 450 mg/kg of body weight. Dosing solutions were analyzed on a routine basis during the study to assure composition and potency. F344/N rats came from Frederick Cancer Research Facility. Rats were quarantined 18 days. Rats were about 61 days old at study initiation. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program. Rats were housed five per cage throughout the study. Feed and water were available ad libitum. Cage racks were rotated every 2 weeks beginning week 37. Clinical observations were made twice daily; findings were recorded at the time of weighing or as necessary. Animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Animals found moribund or surviving to the end of the 2-year studies were killed. Necropsy was performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on rats that died or were killed moribund prior to day 637, on all control and high-dose rats. Selected tissues were examined from all low-dose rats. Histopathology examinations were performed on all grossly visible lesions in all dose groups. The tissues and tissue groups examined are listed in the formal report.

Upon completion of the microscopic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated. All tissues with a diagnosis of neoplasia and all tissues from a randomly selected 10% of the control and high-dose rats and mice were reevaluated microscopically by a quality assessment pathologist. The quality assessment pathologist also examined the following organs: liver,testis and epididymis (male rats).

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides of male and female rat livers; rat testes and epididymis; bones (feet and tail), urogenital tract, and adrenal medulla; examples of disagreements in diagnoses between the laboratory and quality assessment pathologists; and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed.

Statistical Methods: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses, for a possible dose-related effect on survival, used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined.
The primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose.

Result :

In male rats there was no body weight change associated with administration of 112 or 225 mg/kg-day test material. Likewise, there was no apparent adverse effect of the test substance on survival as there was a marginal increase in survival of high-dose males. This was attributed to a marginal decrease in mononuclear cell leukemia in the high-dose males. There were no nonneoplastic toxic lesions or increased incidences in neoplasms in dosed male rats that were attributed to the administration of gamma-butyrolactone in rats.

In female rats there was a reduction in body-weight gain associated with administration of the high-dose (450 mg/kg-day) but not the low dose (225 mg/kg-day). Survival was similar in all female groups. There were no nonneoplastic toxic lesions or increased incidences in neoplasms in dosed male rats that were attributed to the administration of gamma-butyrolactone in rats.

Test substance :

gamma-butyrolactone, CASNO 96-48-0, purity > 97%

Reliability : (1) valid without restriction
Guideline study under glp with no deviations

30.12.2002 (21)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : gavage
Exposure period : 104 weeks
Frequency of treatm. : 5 days a week
Post exposure period :
Doses : 262, or 525 mg/kg of body weight
Result : negative
Control group : yes, concurrent no treatment
Method : other: NTP SOW
Year :
GLP : yes
Test substance :

Method :

Groups of 50 mice of each sex were administered gamma-butyrolactone in corn oil by gavage 5 days a week for up to 103 weeks. Mice of each sex received 0, 262, or 525 mg/kg of body weight. Dosing solutions were analyzed on a routine basis during the study to assure composition and potency. Mice came from Frederick Cancer Research Facility. Mice were quarantined 19 days and at study start, male mice were 55 days old, and female mice were 62 days old. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program. Mice were housed five per cage until week 67 (males) or week 87 (females); after this time mice were housed individually. Feed and water were available ad libitum. Cage racks were rotated every 2 weeks beginning week 37. Clinical observations were made twice daily; findings were recorded at the time of weighing or as necessary. Animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Animals found moribund or surviving to the end of the 2-year studies were killed. Necropsy was performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on rats that died or were killed moribund prior to day 637, on all control, high-dose, and low-dose male mice. Selected tissues were examined from fow-dose female mice. Histopathology examinations were performed on all grossly visible lesions in all dose groups. The tissues and tissue groups examined are listed in the formal report.

Upon completion of the microscopic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated. All tissues with a diagnosis of neoplasia and all tissues from a randomly selected 10% of the control and high-dose mice were reevaluated microscopically by a quality assessment pathologist. The quality assessment pathologist also examined the following organs: adrenal medulla, bone and marrow (female mice).

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides of male mouse skin, bones (feet and tail), urogenital tract, and adrenal medulla; and female mouse ovary and bone marrow; examples of disagreements in diagnoses between the laboratory and quality assessment pathologists; and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed.

Statistical Methods: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not censored. Statistical analyses, for a possible dose-related effect on survival, used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined.
The primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose.

Result :

Mean body weight and survival of high-dose male mice were significantly lower than in controls. High-dose mice were partially sedated or lethargic and inactive shortly after dosing; this seemed to contribute to an increase in fighting related trauma in dosed males and the lower body weights and excess mortality. After the male mice were individually housed (week 67), the difference between mean body weights of dosed and control groups decreased. Body weights of low- and high-dose female mice were lower than that of the controls throughout much of the study, but there was no improvement following the change to individual housing. Survival of dosed female mice was similar to controls. Based on body weight and survival the NOAEL for males was 262 mg/kg and the NOAEL for females was < 262 mg/kg (body weight gain).

Administration of test substance to mice for 2 years was associated with a statistically significant increased incidence of focal hyperplasia of the adrenal medulla in low-dose males but not high-dose males.

There were no nonneoplastic degenerative lesions associated with the administration of gamma-butyrolactone to male or female mice.

There was a statistically significant negative trend for hepatocellular neoplasms in dosed male mice, and the lower incidences in the low- and high-dose groups compared to the controls were significant by survival-adjusted analyses (hepatocellular adenoma or carcinoma combined: 24/50, 8/50, 9/50). Although the lower incidence of hepatocellular neoplasms is associated with the administration of gamma-butyrolactone, it may also be related to the lower body weights of dosed mice.

The incidences of harderian gland adenoma in the dosed groups of male mice were also significantly lower than the incidence in the controls.

Test substance : gamma-butyrolactone, CASNO 96-48-0, purity > 97%

Reliability : (1) valid without restriction

Guideline study under glp with no deviations

30.12.2002 (21)

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Ovulation
Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	i.p.
Exposure period	:	hours
Frequency of treatm.	:	once
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	
Control group	:	
Method	:	
Year	:	
GLP	:	no
Test substance	:	

Method : Mature female Sprague-Dawley rats (Charles River, Cambridge, Massachusetts), 225-250 g in weight were obtained and acclimated to laboratory conditions. They were maintained on a fixed 14-hr light/10-hr dark lighting schedule (lights off 1900 hr). Only those rats exhibiting at least two consecutive 4-day cycles were used for the ovulation studies. Gamma-butyrolactone (Aldrich Chemical Co.) was diluted with saline and injected ip at 1330 hr on proestrus. Sequential blood samples for determination of serum LH and FSH by RIA were taken by substernal heart puncture (0.5-1.0 ml; volume replaced ip by saline) under light ether anesthesia hourly from 1330-1730 hr proestrus. All values for serum LH and FSH were used in data evaluation regardless of whether the animal ovulated. Necropsies were performed on the morning of expected estrus and the degree of ovulation was assessed by counting tubal ova.

Serum LH and FSH levels were determined by RIA from kits supplied by the NIAMDD Rat Pituitary Hormone Program and by Dr. A. Parlow. LH was assayed from duplicate 0.025-ml samples. FSH was determined in duplicate 0.050 ml samples. The lower limit of sensitivity for both hormones was 10 ng/ml.

Result :

The degree of ovulatory inhibition produced by increasing doses of GBL is illustrated in the table. The antiovulatory ED, was approximately 250 mg/kg, which is a subanesthetic dose. At this dose, increase in uterine wet weight accompanied the increased incidence of uterine ballooning, but only the 750-mg/kg dose of GBL produced a significant increase over control. No change was noted in ovarian weight.

Proestrous serum LH levels from the rats described in Table I are illustrated in a figure (not reproduced) that showed GBL produced a significant dose-related decrease in serum LH levels over the time period sampled. Also by 1630 hr, proestrous FSH levels were significantly reduced by doses of GBL above the antiovulatory ED50.

Substance	Dose mg/kg	N	Number rats ovulating	Percentage inhibition of ovulation	Number of ova per ovul'ing rat
Saline -		15	15	0	13.2 ± 0.5*
GBL (ip)	750	5	0	100%	0
	500	7	2	71%	13, 15
	250	11	4	63%	11.7 ± 0.9
	125	5	4	20%	9.5 ± 1.6
	62.5	9	7	22%	12.3 ± 0.7

Test substance :
gamma-butyrolactone, CASNO 96-48-0, From Aldrich Chemical Co, purity not specified.

Conclusion :
Test material inhibits ovulation in rats if injected i.p.at 1330 hour on proestrus. The ED50 is about 250 mg/kg-bw

Reliability : (2) valid with restrictions
Acceptable publication

30.12.2002 (9)

Type : other: Testicular Effects
Species : rat
Sex : male
Strain : Wistar
Route of admin. : drinking water
Exposure period : Not specified
Frequency of treatm. : Daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 0.5, 1 and 2% in drinking water (uncertain)
Control group :

Method :
Male Wistar rats aged 21 days were given free access to tap water containing 1% GBL (n=13). Another group was given tap water containing 2% GBL (n=10). Saccharin was added to improve the taste of the water. Control rats of the same age were given tap water with saccharin (n=12).

In the first attempt there was a significant decrease in the body weight of the rats treated with GBL, a second experiment was performed. Three groups of rats were treated, as were the previous experimental groups; the only difference was that the food given to the rats was carefully controlled, and each group received exactly the same amount of rat-chow pellets. n= 14, 14 and 13 for control, 1% and 2%.

Rats were killed by decapitation and blood was collected from the trunk. Serum was prepared and kept frozen until assayed. Testes and seminal vesicles were dissected free and weighed. Prolactin was assayed in each serum sample by means of the double-antibody radioimmunoassay, described by Niswender et al. [Proc Soc Exp Biol Med 130:793-797(1969)]. Statistical differences between groups was determined using Student's t test.

		Note, duration of dosing not specified, probably around 20 days based on final body weights and specifications of other studies in this publication.
Result	:	In the first part of this experiment, body weight was significantly lower in 1 and 2% GBL-treated rats than in control rats ($p < 0.01$). Testicular weight was also significantly lower in both GBL-treated groups ($p < 0.01$). Serum prolactin was not significantly different in 1 and 2% GBL-treated rats as compared with controls. In the second part of the experiment, body weights were similar in the three groups of rats, whereas testicular weights were significantly lower in 1 and 2% GBL-treated rats ($p < 0.01$). Seminal vesicle weights were not significantly different in any of the three groups of rats. Serum prolactin levels were similar in the control rats and in rats treated with GBL.

##Experiment 1 ##	Control	1% GBL	2% GBL
Mean Body Weight (g)	127.3	107.7	95.0
Testicular Weight (g)	1294	619	447.5
Serum Prolact (ng/ml)	32.7	35.0	21.9
##Experiment 2 ##	Control	0.5%GBL	1.0% GBL
Mean Body Weight (g)	114.2	122.7	118.6
Testicular Weight (g)	995.2	623.5	497.4
Serum Prolact (ng/ml)	22.1	22.5	20.9

		Note: The text and table are duplicated from the original publication. The text states GBL concentrations of 1 and 2% in both experiments, while the table lists 1 and 2 % GBL for experiment 1, and 0.5 and 1% for experiment 2. The testicular weights are also given in "g" although mg is probably the correct units. The uncertainty is compounded by the duration of the "chronic" exposure not being specified.
Test substance	:	gamma-butyrolactone, CASNO 96-48-0, From Sigma Chemical Co, purity not specified.
Conclusion	:	No firm conclusions can be drawn from the data.
Reliability	:	(4) not assignable Cannot be assigned

25.12.2002 (12)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rabbit
Sex	:	female
Strain	:	Himalayan
Route of admin.	:	inhalation
Exposure period	:	days 9 to 17 of gestation
Frequency of treatm.	:	daily
Duration of test	:	6 hours per day
Doses	:	0.5, 1.4 and 5.0 mg/L
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 5 mg/l
NOAEL teratogen.	:	= 5 mg/l
Result	:	negative
Method	:	other: OECD 414, Directive 87/302/EEC, and . EPA-TSCA New and Revised Health Effects Test Guidelines [Developmental Toxicity Study], NTIS, 1984 (EPA 1984)
Year	:	

GLP : yes
Test substance :

Method : The study was carried out based on:
1. OECD Guidelines for Testing of Chemicals, Section 4; Health Effects; Paris 1981, method 414 (OECD 1981)
2. Commission directive 87/302/EEC of November 18, 1987 for 9th adoption of directive 67/548/EEC, pages 24 - 26, 1988 (EEC 1988) and
3. EPA-TSCA New and Revised Health Effects Test Guidelines [Developmental Toxicity Study], NTIS, 1984 (EPA 1984)

Induction of Pregnancy
After an acclimatization period of at least 5 days, the does were fertilized by artificial insemination. The procedure was to administer 0.2 ml of a synthetic hormone that releases LH and FSH from the anterior pituitary lobe (Receptal) by intramuscular injection about 1 hour before insemination. The pooled ejaculate samples used for the artificial insemination were derived from male Himalayan rabbits of the same breed as the females. The day of insemination was designated as day 0 (beginning of the study) and the following day as day 1 post insemination (p.i.).

Animals were individually housed in wire cages with a 12 hour light:dark cycle and fed standard (KILBA) diet and tap water ad libitum except for during the exposures. At the beginning of the study the rabbits were about 18-26 weeks old and weighed about 2.3566 kg. Animals were randomized, based on their body weight, into four groups by means of a computer-generated plan. Animals were identified by ear tattoo

From day 1 p.i. to day 6 p.i., the animals were placed in inhalation chambers for adaptation over 6 hours/day and were exposed to a stream of fresh air, under similar conditions as during exposure. Animals were exposed in the inhalation chambers daily over 6 hours from days 7 - 19 p.i. From day 20 p.i. to the day of sacrifice (day-29 p.i.) the animals were subjected to a post-exposure observation period under the housing conditions above.

The test atmosphere was generated using a two-component atomizer with test substance supplied by a continuous metering pump. The aerosol was generated into an aerosol mixing vessel. In the mixing vessel the aerosol was mixed with conditioned supply air and passed through a cyclonic separator into the exposure chambers.

Animals were exposed to gamma-Butyrolactone vapor and vapor-aerosol-mixtures for 6 hours/day on days 7 to 19 p.i. The target concentrations for the study were set to 0.5 (vapor), 1.4 and 5.0 mg/L (vapor-aerosol-mixture). The animals were treated in whole body inhalation chambers, sitting in specially shielded cages that prevented contamination of their body surface (or a head-nose exposure).

Clinical examination of the animals was performed at least once daily in the pre- and post-exposure period. During the treatment interval, health of the animals was checked before, during and after exposure. Body weight development was followed throughout the study by measuring the mass of the animals on days 3, 7, 10, 13, 16, 19, 21, 24, 27, and 29 p.i.. On day 29 p.i. all animals were sacrificed and the uteri removed. Dams were examined for the following: uterus weight before opening, number of corpora lutea, number and distribution of implants sites. The fetuses were dissected from the uteri, sexed, weighed and examined for any external, soft tissue and skeletal findings.

Fetal examinations:

At necropsy each fetus was weighed and examined macroscopically for any external findings. Furthermore, the viability of the fetuses and the condition of the placentae, the umbilical cords, the fetal membranes and fluids were examined. Individual placental weights were recorded. Soft tissues were examined after the fetuses were sacrificed using carbon dioxide; the abdomen and thorax were opened in order to examine the organs in situ before they were removed. The heart and the kidneys were sectioned to assess the internal structure. Sex of fetuses was determined by internal examination of the gonads.

After skinning, fetuses were fixed in ethyl alcohol. After fixation for 24 hours to 5 days, the fetuses were removed from the fixative for a short while and a cross-section of the heads from all intact fetuses was made in the parietal bone area using a scalpel. The two halves of the heads were carefully bent to allow a thorough examination of the brain. Subsequently, the fetuses were placed back into the fixative for further fixation. If fetal heads indicated severe findings, the heads of these fetuses were severed from the trunk, fixed in BOUIN's solution and later processed and assessed according to WILSON's method. About 10 transverse sections were prepared per head. After the examination the heads treated in this way were discarded.

Skeletal examination of the fetuses: After the soft tissue examination all fetuses were placed in ethyl alcohol for staining of the skeletons according to a modified Dawson method. The stained skeletons were placed on an illuminated plate and examined, evaluated and assessed.

Statistical evaluation of the data was carried out on the Toxicology department computer systems. Data from examination of the dams and fetuses was evaluated with Dunnett's Test (DUNNETT, 1955, 1964) for statistical evaluation of body weight, body weight change, corrected body weight gain (net maternal body weight change), weight of the uterus before it was opened, weight of fetuses, weight of placentae, corpora lutea, implantations, pre- and post-implantation losses, resorptions and live fetuses. Fisher's Exact Test (SIEGEL, 1956) was used for statistical evaluation of conception rate, mortality (of the dams) and all fetal findings.

Result :

Mean concentrations of test substance were 0.50, 1.42 and 5.07 mg/L, averaged over the course of the study, for the low, mid and high-dose groups. Particle size measurement revealed the presence of aerosols at 5.0 mg/l with an MMAD of 2.4 pm and a respirable fraction of 92%. No meaningful partical size data could be generated from the mid-dose concentration.

Clinical Signs indicating adverse reaction to exposure were not seen in any group.

Dam's body and body weight gain for treated groups were comparable to the controls.

Examinations of the dams at termination: At necropsy none of the does of

any test groups showed any substance-induced findings. Only, one spontaneous necropsy finding was recorded for any group. This finding, lungs with edema, was attributed to the sacrifice procedure.
Uterus weights: There were no substantial differences of uterus weights between the controls and test groups. All values lie within the range of biological variation and do not show any relation to treatment.
The conception rate was 100% in all groups.
Concerning all groups, there were no substance-related and/or statistically significant differences in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre and the post-implantation losses, the number of resorptions and viable fetuses. The differences evident are considered to be incidental and within the normal range of deviations for animals of this strain and age as verified by available historical control data from this laboratory.

A summary of some of the pertinent data is given below:

Parameter	cont	low	mid	high
Maternal				
Body wt (kg)	2.71	2.73	2.69	2.69
Preg/mated	15/15	15/15	15/15	15/15
Mat Mortality	0	0	0	0
Corpora leut	7.5	8.3	7.6	8.0
Implant sites	7.1	7.5	7.1	7.6
Preimplantation Loss	6.1	9.2	5.8	5.2
Postimplant Loss	6.1	9.5	10.5	8.3
Resorptions	0.3	0.7	0.7	0.7
Live fetuses	6.7	6.8	6.4	6.9
Males	3.8	2.7	2.9	2.7
Females	2.9	4.1	3,5	4.1
Placental wt	4.3	4.2	4.2	4.1
Fetal weight	39.8	38.7	38.9	37.3
Gross Malformations	0	0	0	0
Gross Variations Fetal incid	1	0	0	3
SKELETAL				
malformations	1	1	2	1
Variations total	25	11	27	23
Variations litters	13	7	13	9
Retardations				
Total	54	70	55	51
Litter	14	14	13	14

Test substance :

gamma-butyrolactone, CASNO 96-48-0, purity = 99.7%

Conclusion :

There were no substantial, substance-related effects on the dams concerning body weight, body weight change, uterine weights, corrected body weight change, clinical and necropsy observations up to and including concentration of 5 mg/l. There were no differences of biological relevance between the control and the substance-treated groups in conception rate, mean number of corpora lutea, total implantations, resorptions and live fetuses, fetal sex ratio or in the values calculated for the pre-and the postimplantation losses.

Although no signs of maternal toxicity were found even at the highest concentration (5 mg/1), no further prenatal inhalation toxicity studies were deemed necessary, because this concentration is in accord with the requirement for the LIMIT TEST, e.g. in the OECD Guideline for testing of chemicals No. 403 (OECD 1981) for acute inhalation studies and the EPA-TSCA guideline "Inhalation developmental toxicity study" § 798.4350 (EPA 1984).

Reliability : (1) valid without restriction

Guideline study under glp with no deviations

Flag : Critical study for SIDS endpoint

30.12.2002 (4)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : day 6 to 15 of pregnancy
Frequency of treatm. : daily
Duration of test :
Doses : 50, 125, 250 or 500 mg/kg-bw
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 500 mg/kg bw
NOAEL teratogen. : = 500 mg/kg bw
Result : Not developmentally toxic under these conditions
Method :
Year : 1988
GLP : no data
Test substance :

Method :

Groups of 10 female pregnant Sprague Dawley rats each received gamma-Butyrolactone in doses of 10, 50, 125, 250 or 500 mg/kg body weight by oral gavage in soybean oil vehicle from 6 to 15 of gestation. A group of 9 animals treated with the solvent served as controls. The solubility of the test material in soybean oil limited the high dose to 500 mg/kg. Body weighs, food and water consumption and clinical sighs were monitored until day 21 when the pups were delivered by Caesarean section. Placental weights, living and dead fetuses, fetal weights, corpora lutea, total resorptions and pre and post-implantation loss were determined. Gross, soft tissue and skeletal examination of the fetuses was conducted.

Remark :

As the maximum dose was controlled by the solubility of the test substance in the vehicle and as there was no maternal toxicity produced, a higher dose-level might have been achieved with an alternate vehicle. This study indicates no developmental hazard up to 500 mg/kg body weight but does not define the maternal or developmental LOAEL. As this dose level is half of the OECD-recommended maximum of 1000 mg/kg in an OECD 414 test, and as there were no adverse effects produced, the material is considered to have little or no developmental hazard based on this study.

Result :

One dam died during treatment in the 125-mg/kg group and three dams died in the 50-mg/kg group. At necropsy, these dams showed signs of lung edema, hyperemia and emphysema. Maternal body weights were not different between control and treated groups and there were not any significant differences in food or water consumption.

Placental weights were not affected in a dose-dependent manner; however, all treated groups showed a reduced placental weight as compared to controls. The number of corpora lutea, total implantations, the living and dead fetuses, total resorptions and pre and post-implantation loss were comparable in control and treatment groups. Some minor skeletal alterations seen in fetuses did not appear systematically and were not attributed to treatment.

The average fetal weight was significantly increased in the 50, 125 and 250 mg/kg bodyweight groups. The authors could not explain the increase in fetal weights.

Test substance : gamma-butyrolactone, CASNO 96-48-0
Reliability : (2) valid with restrictions
Acceptable publication

30.12.2002 (19)

(1) BASF AG, Analytisches Labor; unveroeffentlichte Untersuchung (J.Nr. 119046/02 vom 25.09.1987)

(2) BASF AG, Analytisches Labor; unveroeffentlichte Untersuchung (BRU 86.267)

(3) BASF AG, Labor Oekologie; unveroeffentlichte Untersuchung, (0009/88)

(4) BASF AG, Prenatal Toxicity of gamma-Butyrolactone as a vapor resp. vapor/aerosol mixture in rabbits after inhalation. Project No. 41R0468/91065, sponsored by the BG Chemie. 20 October 1993.

(5) BASF AG, Unpublished result of a BOD study. dated 18.8.77 (1977)

(6) BASF AG, Unpublished result of a fish toxicity study. dated 20.06.77 (1977)

(7) BASF AG, Unpublished results of two algae studies. 1. dated 3.06.1988 ref 2/00w9/88/t96; 2. dated 28.05.1988 ref 2/0009/87/t96

(8) BASF AG: Abt. Toxikologie, unveroeffentlichte Untersuchungen, (X/90), 02.11.1960

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